# SEARCH FOR *E. COLI* O157:H7 AND *SALMONELLA* SPECIES IN WATER SAMPLES AND THEIR ANTIBIOTIC PROFILES FROM SELECTED WELLS IN KATSINA STATE, NIGERIA.

**BY**

**MUKHTAR, Fatima**

**(MSC/SCIEN/39418/2012-2013)**

**A DISSERTATION SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA, IN PARTIAL FULFILLMENT FOR THE AWARD OF A MASTER DEGREE IN MICROBIOLOGY.**

**DEPARTMENT OF MICROBIOLOGY, FACULTY OF SCIENCE,**

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**JUNE, 2016**

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**JUNE, 2016**

## DECLARATION

I hereby declare that the work in this dissertation titled **"SEARCH FOR *E. COLI* O157:H7 AND *SALMONELLA* SPECIES IN WATER SAMPLES AND THEIR ANTIBIOTIC PROFILES FROM SELECTED WELLS IN KATSINA STATE, NIGERIA."** was carried out by me in the Department of Microbiology, under the supervision of Prof. E.D Jatau and Prof. H. I. INABO.

The information derived from the literature has been duly acknowledged and a list of references provided. No part of this work has been presented for another degree or diploma elsewhere.

…….. …….

Mukhtar Fatima. Date

## CERTIFICATION

This dissertation, titled **"SEARCH FOR E. *COLI* O157:H7 AND *SALMONELLA* SPECIES IN WATER SAMPLES AND THEIR ANTIBIOTIC PROFILES FROM SELECTED WELLS IN KATSINA STATE, NIGERIA."** meets the regulations governing the award of Master of Science Degree in Microbiology of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

Prof. E.D Jatau ……...
Chairman, Supervisory Committee, Date
Department of Microbiology,
Ahmadu Bello University, Zari

Prof. H. I. INABO. …….
Member, Supervisory Committee, Date
Department of Microbiology,
Ahmadu Bello University, Zaria

Prof. I.O. Abdullahi …….
Head, Department of Microbiology, Date
Ahmadu Bello University, Zaria

Prof. Kabir Bala ……..
Dean, School of Post Graduate Studies, Date
Ahmadu Bello University, Zaria

## DEDICATION

This dissertation is dedicated to my parents, without whom I could not have been here, and my two little daughters Aysha and Ummulkhair. May Allah guide and protect you were ever you are

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## ABSTRACT

The lack of clean water resources and sanitation facilities looms as one of the most serious environmental health problems faced by a large fraction of the world's population. The onset of water borne diseases is enormous and largely attributed by the fact that, the World Health Organisation (WHO) has estimated that 1.1 billion people globally lack basic access to drinking water resources,while 2.4 billion have inadequate sanitation facilities which accounts for many water related acute and chronic diseases. The research hyphothesis is that, *E.coli* O157:H7 and *Salmonella* spp are more likely to be found in wells with close proximity to pit laterines. The study was designed to search for the presence of *Escherichia coli* O157:H7 and *Salmonella* spp from wells in Dan-musa, Mani and Danja Local Government Areas of Katsina State. The antibiotics susceptibility profiles of the isolates was also carried out. Some physicochemical parameters (temperature, pH, turbidity and odour), and also distance of wells to pit laterines were determined at collection points. The organisms were isolated by cultural methods using selective media and were subjected to series of biochemical tests. *Salmonella* isolates were sero-grouped using seroquick grouping reagents. Antibiotic susceptibility testing was performed by disk diffusion method. The physicochemical parameters analysed were within the WHO range but all the distances measured were below the set standard of 30 meters by WHO and the Nigerian Environmental Protection Agency. Dan-musa Local Government Area had the highest mean coliform and aerobic plate counts of 20.7cfu/ml and 3.47±0.02 respectively, while Danja had least mean coliform and aerobic plate count of 13.8cfu/ml and 1.99±0.02. Out of the 300 well water samples collected, a total of 246 presumptive *E.coli* isolates were isolated and 7 out of them were serologically confirmed to be *E.coli* O157:H7, also a total of 11 *Salmonella* strains were isolated belonging to three(3) different sero-groups (Group D, A and B). Significant

statistical association was found between the occurrence of these bacterial isolates and distance of wells to pit laterines. All the isolates were resistant to multiple classes of antibiotics, the highest resistance of 100% was to tetracycline and augmentin by *E.coli* O157:H7, while for *Salmonella* species, highest resistance was to ceftazidime and cefuroxime, 100% each. However, the two organisms were sensitive to flouroquinolones and all the *Salmonella* isolates were susceptible to chloramphenicol and cefoxitin. The presence of these pathogenic bacteria from the well water samples analysed connote a serious health risk to consumers due to threat of disease and antibiotic resistance. This study accenuates the need to set standards for citing of wells away from pit laterines in the study locations, Provision of adequate potable water, improved sanitation and restricting the use of drugs either for self-medication and as growth promoters in food animals were recommended to overcome the problems of multi-drug resistant organisms.

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# CHAPTER ONE

# 1.0 INTRODUCTION

## 1.1 BACKGROUND OF STUDY

Waterborne diseases due to faecal pollution of human and animal origin, are responsible for approximately 2.2 million deaths annually in children under the age of five years in developing countries (WHO, 2002a; WHO, 2002b). Most of these deaths are due to inadequate potable water supplies, poor hygiene practices and insufficient sanitation infrastructures (Sobsey, 2002; WHO, 2002a; WHO 2002b). The World Health Organization (WHO) estimated that 1.2billion of the world's population lack access to safe drinking water and these people use any source of water, usually the most convenient source, regardless of its quality (WHO, 2002a).

The United Nations (UN) set a goal in their Millennium Declaration to reduce the amount of people without safe drinking water by half in the year 2015 (UN, 2000). Safe drinking water for human consumption should be free from pathogens such as bacteria, viruses and protozoan parasites, meet the standard guidelines for taste, odour, appearance and chemical concentrations, and must be available in adequate quantities for domestic purposes (Kirkwood, 1998).

However, inadequate sanitation and persistent faecal contamination of water sources is responsible for a large percentage of people in both developed and developing countries not having access to microbiologically safe drinking water and for suffering from diarrhoeal diseases (WHO, 2002a; WHO, 2002b) Diarrhoeal diseases are responsible for approximately 2.5 million deaths annually in developing countries, affecting children

younger than five years, especially those in areas devoid of access to potable water supply and sanitation (Kosek *et al.,* 2003; Obi *et al.,* 2003; Lin *et al*., 2004; Obi *et al.,* 2004).

Pit latrine is a common method of excreta disposal in the developing world. It is popular and widely used in urban slums as well as rural areas probably because it is the simplest, cheapest and the most efficient excreta disposal method that is within the reach of poor people. One of the major contributing factors of groundwater pollution is pit latrine mostly located near water sources such as shallow wells and boreholes. In fact, pit latrines have been identified as the major source of contamination of wells with faecal matter (Pritchard *et al.,* 2007).

Many infectious diseases are associated with faecally contaminated water and are a major cause of morbidity and mortality worldwide (Leclerc *et al.,* 2002; Theron and Cloete, 2002). Waterborne diseases are caused by enteric pathogens such as bacteria, viruses and parasites that are transmitted by the faecal oral route (Leclerc *et al.,* 2002; Theron and Cloete, 2002).

Waterborne spread of infection by these pathogenic microorganisms depends on several factors such as: the survival of these microorganisms in the water environment, the infectious dose of the microorganisms required to cause a disease in susceptible individuals, the microbiological and physico-chemical quality of the water, the presence or absence of water treatment and the season of the year (Leclerc *et al.,* 2002; Theron and Cloete, 2002).

## 1.2 *ESCHERICHIA COLI*

*Escherichia coli* is a common member of the intestinal microflora of both humans and warm blooded animals. It is a commensal or opportunistic pathogen implicated in acute urinary tract infections (UTI) and gastrointestinal tract infections (Swiecicka *et al.*, 2003). It is a consistent and predominant facultative inhabitant of the human gastro-intestinal tract, thus its regular presence in the intestine and faeces of warm blooded animals makes the bacterium an indicator of faecal pollution (Yang *et al*., 2004).

*Escherichia coli* is one of the predominant species of facultative anaerobes in the human gut and usually harmless to the host. However, a group of pathogenic *E. coli* has emerged that causes diarrhoeal disease in humans known as Diarrhoeagenic *E. coli* (Szakal *et al*., 2001) or commonly known as pathogenic *E. coli*. These groups are classified based on their unique virulence factors and can only be identified by these traits. Hence, analysis for pathogenic *E. coli* often requires that the isolates be first identified as *E. coli* before testing for virulence markers. The pathogenic groups include enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and perhaps others that are not yet well characterized (Szakal *et al*., 2001). Of these, only the first 4 groups have been implicated in food or water borne illness.

EHEC is recognized as the primary cause of haemorrhagic colitis (HC) or bloody diarrhoea, which can progress to the potentially fatal hemolytic uremic syndrome (HUS). EHEC is typified by the production of verotoxin or Shiga toxins (*Stx*). Although *Stx*1 and *Stx*2 are most often implicated in human illness, several variants of Stx2 exist. There are

many serotypes of *Stx*-producing *E. coli* (STEC), but only those that have been clinically associated with HC are designated as EHEC. Of these, O157:H7 is the prototypic EHEC and most often implicated in illness worldwide (CDC, 1993). The infectious dose for O157:H7 is estimated to be 10 - 100 cells, EHEC infections are mostly food or water borne and have implicated undercooked ground beef (CDC, 1993). raw milk, cold sandwiches and water (Swerdlow *et al.,* 1992). Unpasteurized apple juice, sprouts and vegetables (Combo-Sebbati *et al.,* 1997). EHEC O157:H7 is phenotypically distinct from *E. coli* in that they exhibit slow or no fermentation of sorbitol and do not have glucuronidase activity.

*Escherichia coli* O157:H7 which is a food borne pathogen has been associated with meat, meat-produce, unpasteurized milk, and vegetables generally fertilized with contaminated cow manure, juice and water-related disease outbreaks (Campbell *et al.,* 2001). This pathogen, which is known for its low infective dose and its ability to cause severe disease and death, emerged as a foodborne threat in the 1980s and early 1990s (Mead, 1999). Food-borne outbreaks associated with *E. coli* O157:H7 have been well documented worldwide. Infections due to *E. coli* O157:H7 can result in severe bloody diarrhoea (haemorrhagic colitis) and in life threatening complications such as (HUS) haemolytic uraemic syndrome (Kumar *et al.,* 2001).

## 1.3 *E. COLI* AS AN INDICATOR OF FAECAL POLLUTION

Globally *E. coli* is used as the preferred indicator of faecal pollution (Edberg *et al.,* 2000). It is a Gram negative bacterium and predominantly an inhabitant of the intestines of warm blooded animals and humans, which is used to indicate recent faecal pollution of water samples (Edberg *et al.,* 2000). Confirmation tests for *E. coli* include testing for the presence of the enzyme β-glucuronidase, Gram staining, absence of urease activity, production of acid and gas from lactose and indole production (Rice *et al.,* 1991; Standard Methods, 1995).

Commercially available growth media containing the fluorogenic substrate 4-methyl-umbelliferyl-β-D-glucuronidase (MUG) is used for the isolation and identification of *E. coli* from water samples (Shadix and Rice, 1991; Covert *et al.,* 1992). The *E. coli* bacteria hydrolyse the MUG in the media, which then fluoresces under ultraviolet light (Shadix and Rice, 1991; Covert *et al.,* 1992). However, false negative results on this media have been found due to injured cells, lack of expression of the gene which codes for the enzyme β-glucuronidase by the *E. coli* bacterium isolate, and non-utilization of the MUG reagent in the media by some *E. coli* strains (NRC, 2004).

## 1.4 *SALMONELLA*

*Salmonella* is an enteric pathogen with a worldwide distribution that comprises a large number of serovars characterized by different host specificity and distribution. This microorganism is one of the leading causes of intestinal illness throughout the world as well as the etiological agent of more severe systemic diseases such as typhoid and paratyphoid fever. Zoonotic *Salmonellae* are commonly described as foodborne

pathogens, however, drinking water as well as natural waters are known to be an important source for the transmission of these enteric microorganisms (Ashbolt, 2004).

*Salmonella*, just like other enteric bacteria, is spread by the fecal–oral route of contamination. This microorganism can enter the aquatic environment directly with faeces of infected humans or animals or indirectly, e.g., via sewage discharge or agricultural land run off. Overall, *Salmonella* species and subspecies can be found in a large variety of vertebrates. Beside humans, animal sources of *Salmonella* include pets, farm animals and wild animals; calves, poultry, pigs, sheep as well as wild birds (sea gull, pigeon) and reptiles can all be reservoirs of *Salmonella* (Wray & Wray, 2000). Plants, insects and algae were also found capable of harboring *Salmonella* and might be implicated in the transmission of this enteric pathogen (Byappanahalli *et al*.,2009). *Salmonellae* are frequently found in environmental samples. They are usually present in large numbers in raw sewage ($10^3$–$10^4$CFU/L) and can still be present in wastewater effluent after advanced secondary treatment including coagulation, filtration and disinfection (Maier *et al*., 2000). Soils and sediments were also found to harbor *salmonellae* (Abdel-Monem & Dowidar, 1990), sediment particles are believed to function as a micro ecological niche enhancing *Salmonellae* survival in lakes (Chandran *et al.,* 2011). In the aquatic environment this pathogen has been repeatedly detected in various types of natural waters such as rivers, lakes, coastal waters, estuarine as well as contaminated ground water (Wilkes *et al.,* 2009). In addition to its widespread occurrence, elevated survival capacities in non-host environment have been reported for *Salmonella* (Winfield & Groisman, 2003). The growth of *Salmonella* in non-host environments such as wastewater sludge and compost has also been reported (Zaleski *et*

*al.*, 2005) and the growth of *Salmonella* in water supplies is also considered possible, due to its ability to colonize surfaces and replicate in biofilms of distribution system pipes (Jones & Bradshaw, 1996). However, standard disinfection procedures used in drinking water treatment processes are active against *salmonellae* (Cicmanec *et al.*, 2004).

Taxonomically the genus *Salmonella* comprises two species namely *S.* bongori and *S.* enterica. The species *S.* enterica is further differentiated in to six subspecies (Enterica, Salamae, Arizonae, Diarizonae, Indica and Houtenae) among which the *S.* enterica subspecies enterica is mainly associated to human and other warm blooded vertebrates. Traditionally members of the genus *Salmonella* are clustered in serovars according to their flagellar (H) and somatic (O) antigens. Currently over 2,400 *Salmonella* serovars have been described but only about 50 serovars, all within the subspecies enterica are common causes of infections in humans and warm blooded animals (Popoff, 2001). *Salmonella* serovars have different host specificity, diverse geographic distribution, and cause different syndromes. On the basis of the clinical syndromes caused, *Salmonella* are divided in to two distinct groups namely the typhoidal and non- typhoidal *Salmonella* serovars (Pond, 2005).

Enteric fevers, typhoid and paratyphoid fever are severe, contagious systemic diseases caused by the infection of the serovars Typhi and Paratyphi. Even though not common in developed countries, enteric fevers remain an important and persistent health problem in less industrialized nations. Overall, in 2003 an annual incidence of approximately 17 million cases of typhoid and paratyphoid fevers was reported worldwide (Kindhauser, 2003). Differently from other *Salmonella* serovars, Typhi and Paratyphi are host adapted

and can only infect humans; stools of infected persons are therefore the original source of contaminations for these pathogens. Water contaminated with faeces of human cases and carriers is one of the main vehicles of typhoid fever infections. Differently from typhoidal *Salmonella* strains, non-typhoidal *Salmonellae,* the ubiquitous subtypes found in a number of animal species, are more frequently associated to foodborne than to water-borne transmission. These zoonotic *Salmonella* serovars tend to cause acute but usually self-limiting gastroenteritis. In some patients, however, these same serovars can cause severe systemic diseases such as osteomyelitis, pneumonia and meningitis (Pond, 2005).

Severe and invasive presentations of non-typhoidal salmonellosis in Africa are common in children with co-morbidities (Graham, 2002) and immune compromised HIV infected adults (Gordon, 2008). Notably the emergence of MDR strains of *Salmonella* Enteriditis and Typhimurium was associated with the observed incidence of invasive salmonellosis (Gordon &Graham, 2008).

## 1.5 STATEMENT OF PROBLEM

*Escherichia coli* O157:H7 and *Salmonella* species have become a significant public health problem world-wide (Armstrong *et al*., 1996). The modes of transmission include animal-to person, foodborne, waterborne and person to person (Coia, 1998). Enterohaemorrhagic strains of *Escherichia coli*, especially (*E. coli* O157:H7) have emerged as important enteric pathogens in recent years. Various serotypes have been implicated in human disease, but O157:H7 is the most common. Cattle dung has been recognized as the principal reservoir of the microorganisms in waterborne and food-borne outbreaks and sporadic infections (Armstrong *et al.*, 1996; Coia, 1998).

Enterohaemorrhagic *E. coli* (EHEC) O157:H7 is the dorminant Shiga toxin producing strain that is known to be associated with both outbreak and sporadic cases of human diseases ranging from uncomplicated diarrhoea to haemorrhagic colitis and haemolytic uraemic syndrome (HUS). Their ability to cause severe diseases is related to their capacity to secrete Shiga toxins also called verotoxins. Typhoidal salmonellosis and non-typhoidal salmonellosis are major health problems in developing countries to which Nigeria belongs. The problems, according to many findings are associated with unhygienic processing and preparing of foods, substandard water supply, inadequate sanitary measures and emergence of multidrug resistance in some *Salmonella* strains.

In Nigeria, particularly Katsina especially in the rural and sub-urban communities, water for drinking and other domestic uses is mostly obtained from wells dug by inhabitants (Oyetao *et al*., 2007). This is in addition to the water available in streams and rivers in rural communities. The reliance on untreated ground water (wells) and streams is due to the lack of pipe borne water. Such wells and streams are subject to contamination with pathogenic bacteria because of their proximity to human activities and latrines, also the presence of cattles and other ruminants who are reservoir of these pathogenic bacteria in their homes (Moniruzzaman *et al.*, 2011). Poor wastewater and solid waste management, poor construction and inadequate protection of the wells and proximity of latrines to the wells predispose the water to faecal contamination (Eedema *et al*., 2001). Natural groundwater is usually of good quality, but this can deteriorate due to inadequate source protection and poor resource management. Mechanisms of groundwater recharge and the natural attenuation capacity also depend on soil type and geomorphologic characteristics (Oyedeji *et al.*, 2010; Osho *et al.*, 2010).

The prevalence of diseases such as diarrhoea, typhoid fever, cholera and bacillary dysentery among the populace has been traced to the consumption of unsafe water and unhygienic drinking water (Mead *et al.,* 1999). The consumption of drinking water contaminated with pathogenic microbes of faecal origin is a significant risk to human health in the developing world, especially in remote rural areas and peri-urban `shanty' communities. Over 3 million deaths per year are attributed to waterborne diarrhoeal diseases, especially among infants and young children in poor communities in Africa, Asia and South-America (WHO, 2008).

The most dangerous form of water pollution occurs when faecal contaminants enter water supplies and sources. Pathogens such as *Salmonella* species, *Shigella* species, *Vibrio cholerae* and *E. coli* that are shed in human and animal faeces ultimately find their way into water supplies through direct and indirect deposition of faecal contaminants into the wells either by humans, animals and probably some soil borne pathogens blown by wind into the wells (Dipaola, 1998).

Enteric pathogens are typically responsible for several waterborne sicknesses (Sabae, 2004). Contamination of water is a serious environmental problem as it adversely affects human health and the biodiversity in the aquatic ecosystem. The use of indicator bacteria such as faecal coliforms (FC) in water quality determination on fresh water source is widely used (You-Joe *et al.,* 2008). Water borne diseases are one of the major public health concerns in developing countries (Cheesbrough, 2000). It is one of the leading causes of mortality and morbidity in all age groups particularly children below 5years and immunocompromised individuals.

## 1.6 JUSTIFICATION

The quality of water is of importance to human and animal health (WHO, 2010). Poor water quality continues to be a leading cause of health problems especially in developing countries (WHO, 2010).

Water of good drinking quality is of basic importance to human physiology and man's continued existence depends very much on its availability. Safety and quality of drinking water is always an important public health concern (Moniruzzaman *et al.*, 2011). The provision of potable water to the rural and urban population is necessary to prevent health hazards (Lemo, 2012). Before water can be described as potable, it has to comply with certain physical, chemical and microbiological standards, which are designed to ensure that the water is palatable and safe for drinking (Nataro *et al*., 1998). Potable water is defined as water that is free from disease producing microorganisms and chemical substances deleterious to health. Water borne diseases continue to be one of the major health problems especially in developing nations particularly in rural and sub-urban areas in Katsina State.

Nigeria has been ranked as one of the countries without access to improved water sources (WHO, 2008). Isolation of *E. coli* O157:H7 and *Salmonella* species from water sources connote a serious health risk to consumers due to the threat of disease and antibiotic resistance which in turn reduces treatment options (Willis *et al*., 2000).

## 1.7 AIM AND OBJECTIVES

The aim of this research work is to determine the antibacterial susceptibility profiles of *E. coli* O157:H7 and *Salmonella* species from wells in some selected local governments of Kastina State.

## 1.8 SPECIFIC OBJECTIVES

1. To determine the mean coliform counts and total aerobic plate count of the well water samples in the study area.
2. To determine some physicochemical parameters (pH, Temperature, Turbidity and Odour) of the well water samples in the selected local government areas.
3. To isolate and characterize biochemically and serologically, *E. coli* O157:H7 and *Salmonella* species from wells.
4. To determine the antibacterial susceptibility profiles of *E. coli* O157:H7 and *Salmonella* species using Disk Diffusion Method.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

## **2.1** WATER

**About 2.5 billion people, roughly 40% of the world population lack access to safe drinking water (Salaam, 2012). This teeming population of people are at risk of contracting water borne diseases, the most susceptible being children, the elderly, pregnant women and immunocompromised individuals. This makes waterborne illnesses one of the five leading causes of death among children under age five (Hunter, 2003). About 40% of deaths in developing nations occur due to infections from water related diseases and an estimated 500 million cases of diarrhea, occurs every year in children below 5 years in parts of Asia, Africa and Latin America (WHO, 2008; Adejuwon, 2011). In Nigeria, drinking water pollution is further exacerbated due to the alarming rate of urbanization as major cities reportedly grow at rates between 10- 15% per annum (Yusuf, 2007) and thus, human activities including soil fertility remediation, indiscriminate refuse and waste disposal, and the use of septic tanks, soak-away pits and pit latrines are on the increase. Groundwater pollution has been attributed to the process of industrialization and urbanization that has progressively developed over time without any regard for environmental consequences which eventually results in the deterioration of physical, chemical and biological properties of water (Isikwue *et al.,* 2011)**

### 2.1.1 *Quality of ground water*

**Physically, ground water is generally clear, colourless, with little or no suspended matter and has a relatively constant temperature. It is also generally free from the very minute organisms which cause diseases. This is another benefit that results from the slow filtering action provided as the water flows through the ground. Also the lack of oxygen and nutrients in the ground water makes it an unfavorable environment for disease producing organisms to grow and multiply.**

**The chemical quality of ground water is also considerably influenced by its relative slow rate of travel through the ground. According to Itah and Akpan (2005), ground water is known to be purer than the surface water. Unfortunately it becomes chemically contaminated by pesticides, herbicides and fertilizers which have been a boom to the world farmers, but a bomb to the earth's water. Changes in borehole water quality may be due to ground water pollution. Water pollution is the modification of the physical, chemical and biological properties of water restricting or preventing its application (Sax, 1994).**

**Ground water pollution arises when leachate or pollutant percolates through the soil down to the phreatic surface. Once the leachate reaches the ground water, the path way of these contaminants is that of ground water flow, and factors such as permeability of the cover deposits, depth of impermeable layers and hydraulic head gradient in the aquifer become important. The depth of penetration in the aquifer is also dependent on the distance from the nearest ground water divide, and from the nearest drainage channel (Eja, 2002). According to the author, the pollutants may be introduced in a natural way e.g. through rain water, sea water, earth crust and biological activities. Pollutants introduced by human activities may come from point sources and non-point sources. Pollution originating from farming practice, the application of agro chemicals and the use of sewage sludge as fertilizer supplements represents the point sources. In contrast, point sources of pollution are differentiated primarily from the non-point sources by the precision with which the source of contamination can be identified e.g. the discharge of wastes to landfill sites, the surface injection of wastes via deep boreholes and the disposal of effluents by irrigation to land constitute the major persistent sources of point pollution.**

**The main factors that influence the deterioration of water quality are agricultural wastes, domestic and municipal sewage, commercial and industrial wastes. Also the various water pollutants known are derived from the afforested factors and are enumerated to include organic and inorganic materials, salts, nutrients, heavy metals, radioactive wastes, pesticides, pathogens, oil and heat (Ademoroti, 1996). Other sources and types of contaminants include widely used substances such as highway salt, fertilizers that are spread across the land surface.**

### 2.1.2 *Shallow wells as source of drinking water*

**The use of shallow ground water sources for drinking and other domestic purposes is a common feature for many low income communities in developing countries (Adejuwon, 2011). Ground water which occurs beneath the earth surface is considered free from contamination, hence usable but anthropogenic as well as natural factors are affecting the quality as well as quantity of this valuable resource (Eja, 2002). It has been estimated that once pollution enters the subsurface environment, it may remain concealed for many years, becoming dispersed over wide areas of groundwater aquifer and rendering groundwater supplies unsuitable for consumption and other uses. Therefore, understanding the potential influences of human activity on ground water quality is important for protection and sustainable use of ground water resources (Jehangir *et al.,* 2011). Shallow wells are normally located in the valleys where the ground water table is relatively high (1-4m below ground level) and infiltration of rain and river water plays a main part in groundwater recharge (Pritchard *et al.,* 2008).**

**Ground water contamination is the result of polluted water infiltrating through the soil and rock and eventually reaching the ground water. This process might take many years and might take place at varying distances from various wells where such contaminations are found. Once the ground water is contaminated, it is very difficult to remediate. No doubt that the new technologies will always reduce the pollution level (Geetha *et al.,* 2008). Human health, agricultural development and the ecosystems are all at risk unless water and land systems are effectively managed (Kehinde *et al.,* 2009).**

**Pollution of ground water refers to any deterioration in the quality of the water resulting from the activities of man. This definition also includes apparently natural processes like saltwater encroachment into freshwater-bearing aquifers in coastal areas resulting from the artificial lowering of ground-water heads (Eja, 2002). Most pollution of ground water results from the disposal of domestic, municipal and industrial wastes on the land surface, in shallow excavations including septic tanks, or through deep wells and mines; the use of fertilizers and other agricultural chemicals; leaks in sewers, storage tanks, pipelines; and animal feedlots (Eja, 2002). Efe (2008) stated that the longer the polluted water travels through the soil formation the better (cleaner) it becomes. Essien (1996) stated that shallow wells close to sites that are contaminated will invariably be contaminated with the contaminating substances if they are less than 15m deep. Studies by Bolaji and Martins (2008) in Abeokuta, Western Nigeria showed that the microbial quality of water from 24 wells varied with depth and nearly all of the wells were faecally contaminated (Kehinde *et al.,* 2009).**

**The magnitude of a pollution problem depends on the size of the affected area, the amount of the pollutant involved, the solubility, toxicity, and density of the pollutant, the mineral composition and hydraulic characteristics of the soils and rocks through which the pollutant moves, and the effect or potential effect on ground-water use (Kehinde *et al.,* 2009).**

**Due to the increase in population growth and elevated living standards and coupled with the ever increasing demands for clean water around the world, more water is required for growing environmental concerns such as aquatic life, wildlife, scenic values, and riparian habitats (Li *et al.,* 2007). The upsurge in population and the establishment of industries involved in the manufacture of various agrochemicals, petrochemical and house-hold products have resulted in increase in the production of hazardous substances including heavy metals in developing countries (Oguzie and Okhagbuzo, 2010). Application of various agrochemicals and fertilizers for improved and increased agricultural produce can release contaminants of various categories such as nitrate, bacteria, viruses, and hazardous household chemicals to the subsurface, posing potential threats to nearby wells and surface water(Kehinde *et al.,* 2009). The impact of human activities in and around agricultural farmlands is felt on the physical and chemical properties of water on which the sustenance of the various forms of life is dependent.**

**For most communities, the most secure source of safe drinking water is pipe-borne water from municipal water treatment plants. Often, most of water treatment facilities do not deliver or fail to meet the water requirements of the served community; due to corruption, lack of maintenance or increased population (Oguzie and Okhagbuzo, 2010). The scarcity of piped water has made communities to find alternative sources of water: ground water sources being a ready source. Wells are a common ground water source readily explored to meet community water requirement or make up the short fall (Adekunle, 2009).**

**The lack of safe drinking water and adequate sanitation measures lead to a number of diseases such as cholera, dysentery, salmonellosis and typhoid, and every year millions of lives are claimed in developing countries. Diarrhoea is the major cause of death of more than 2 million people per year world-wide, mostly children under the age of five. It is a symptom of infection or the result of a combination of a variety of enteric pathogens (Zamxaka *et al*., 2004).**

### 2.1.3 *Water quality standards*

**The incidents of water borne disease and epidemics nation wide arising from drinking water of doubtful quality have become of great concern. The primary purpose of the guideline for drinking water quality is the protection of public health (WHO, 2006). As described by (Mishra and Bhatt 2007), water quality standard is a measure, principle or rule established by authority set to protect the water resource for uses such as drinking water supply, recreational uses and aesthetics, agriculture (irrigation and livestock watering), protection of aquatic life and industrial water supplies. In order to maintain water quality, guidelines for drinking water was set up by the World Health Organisation (Oguzie and Okhagbuzo, 2010). A guideline value represents the level (a concentration or number) of a constituent that ensures aesthetically pleasing water and does not result in any significant risk to the health of the consumer (WHO, 2007).**

### 2.1.4 *Quality of drinking water*

**In general, certain requirements must be met for water to be fit for human consumption. If these requirements are met, the water can be described as wholesome, potable or simply water supply. According to Eja (2002), the requirements are:**

**(i) Freedom from microorganisms and chemical substances which might be injurious to health. This is the most important requirement.**

**(ii) Drinking water should be of such composition that consumers do not question the safety of the water. This requirement implies that turbidity, colour, taste and odour should be low. Macro organisms (e.g. worms, aquatic and fly nymphs) should be absent.**

**(iii) Drinking water should be suitable for housekeeping and for this reason, iron and manganese content should be low, because these substances colour laundry (like shirt) during washing. Iron causes a brown colour, while manganese causes a black colour. Hardness should be low, because water with a high hardness causes scale formation in water heaters by precipitation of calcium carbonates. Moreover, a high hardness implies that a high dosage of detergents is required for washing.**

**(iv) Drinking water should not be aggressive to materials such as lead, copper, asbestos, cement and concrete, cast iron, galvanized steel, PVC (Polyvinylchloride) and PE (Polyethylene). This is because pipes, tubes**

**and apparatus used in distribution systems and plumbing installations may consist of these materials. Pipes, tubes and apparatus affected by water cause many problems.**

### 2.1.5 *Contamination of wells*

**Shallow pumping wells can often supply drinking water at a very low cost, but because impurities from the surface easily reach shallow sources, a greater risk of contamination occurs to these wells when they are compared to deeper wells (Priis-Uston, 2004). Contamination of the wells increases during the rainy seasons where the aquifer is "topped up" more rapidly and both vertical and horizontal migrations of water are accelerated (Morgan, 1990).**

**Most of the bacteria, viruses, parasites, and fungi that contaminate well water come from faecal material of humans and other animals (Dillon, 1997). Common bacterial contaminants include *E. coli, Salmonella, Shigella* and *Campylobacter jejuni*. Viral contaminants include Norovirus, Sapovirus, Rotavirus, Enteroviruses and Hepatitis A and E. Parasites include *Giardia lamblia, Cryptosporidium, Cyclospora cayetanensis*, and *Microsporidia*. These pathogenic organisms are the most important sources of serious illnesses and deaths especially among young children in poor countries. Water related diseases such as cholera, bacillary dysentery, typhoid, hepatitis A, and diarrhea are feco-oral in their transmission (Priis-Uston, 2004).**

**The quality of the well water can be significantly increased by lining the well, sealing the well head, fitting a self-priming hand pump, constructing an apron, ensuring the area is kept clean and free from stagnant water and animals.**

### 2.1.6 *Physico-chemical assessment of water*

**Physico-chemical parameters are physical and chemical parameters associated with water which have an influence on its quality and which also affect the biological constituents of the water (Oluyemi *et al.,* 2010). The physical factors such as temperature, turbidity, colour etc. can affect the aesthetics and taste of the water and may complicate the removal of microbial pathogens during water treatment. The chemical parameters include pH and anions (Oluyemi *et al.,* 2010).**

### *2.1.6.1 pH*

**The pH of a solution is a measure of its acidity or alkalinity, and normally dependent upon the activity of hydrogen ions. It indicates the intensity of the acidic or basic character of a solution and is controlled by the dissolved chemical compounds and biochemical processes in solution (Anon, 1996). In general, a pH less than 7 is considered acidic, soft and corrosive. A pH greater than 7 is considered basic. Changes in pH can indicate a change in water quality. Heavy metals such as cadmium, lead and chromium dissolves more easily in acidic water. This is important because many heavy metals also become much more toxic when dissolved in water. WHO (2008) guidelines suggest that the optimum pH required in drinking water should be in the range 6.5-8.5.**

### *2.1.6.2 Temperature*

**Temperature is the measure of how much heat is present in the water. It is desirable that the temperature of drinking water should not exceed 15$^{o}$C because the palatability of water is enhanced by its coolness. Low water temperatures offer a number of benefits. A temperature below 15$^{o}$C will tend to reduce the growth of nuisance organisms and hence minimise associated taste, colour, odour and corrosion problems (Oluyemi *et al.,* 2010).**

### *2.1.6.3 Turbidity*

**Turbidity is a measure of the amount of light scattered by suspended particles and can be considered as the "cloudiness" of water sample (Oluyemi *et al.,* 2010). The higher the total suspended solids in the water, the higher the turbidity. It is influenced by sediments from erosion, urban run-offs, re-suspended sediments from the bottom and phytoplankton. (Morgan, 1990).**

**Turbidity is measured in Nephlometric Turbidity Units (NTU). The maximum allowed turbidity in drinking water established by the (WHO, 2004) should not be more than 5 NTU but ideally a value below 1 NTU is desired. Turbidity limits transmission of sunlight thereby reducing photosynthesis of aquatic plants. Although it does not adversely affect human health, turbidity is an important parameter in that it can protect microorganisms from disinfection effects, can stimulate bacteria growth and indicates problems with treatment process (WHO, 2004).**

## 2.2 *ESCHERICHIA COLI*

***Escherichia coli* was first described in 1885 by Theodor Escherich (Escherich, 1988). Escherich, a Bavarian paediatrician, had performed studies on the intestinal flora of infants and had discovered a normal microbial inhabitant in healthy individuals, which he named Bacterium coli commune. In 1919, the bacterium was renamed in his honour to *Escherichia coli* (Kaper, 2004).**

### 2.2.1 *Morphology of E.coli*

**The species *E. coli* comprises gram-negative, oxidase-negative straight cylindrical rods measuring 1.1-1.5 x 2.0-6.0 µm. They are aerobic and facultative anaerobic, rendered motile by peritrichous flagella, or non-motile (Scheutz & Strockbine, 2005).**

**The taxonomy of *E. coli* is summarized below:**

**Phylum: Proteobacteria**

**Class: Gamma-proteobacteria**

**Order: Enterobacteriales**

**Family: Enterobacteriaceae**

**Genus: *Escherichia***

**Species: *Escherichia coli* (VetBakt, 2007)**

### 2.2.2 *Physiological characteristics of E.coli O157:H7*

**_Escherichia coli_ O157:H7 possess metabolic characteristics distinct from other _E. coli_. Most isolated strains are unable to ferment sorbitol within 24hours and cannot hydrolyze 4-methylumbrelliferyl-D-glucuronide (as lacking β-glucuronidase enzyme) (Strockbine _et al.,_ 1998). These two characteristics, together with the inability to grow well at temperatures > 44.5 °C are very important in discriminating between O157:H7 and other _E. coli._ The antigenic composition of outer cell membrane is the most important characteristic that leads to identification of this serotype. _E. coli_ O157:H7 have a cell envelope structure typical of Gram-negative cells, and thus, posses an outer membrane with a lipopolysaccharide component that is distinct from the cytoplasmic membrane.**

**The O157 antigen is defined by the carbohydrate composition and structure within the lipopolysaccharide. The H7 antigen is determined by the unique polypeptide composition of the flagella. (Strockbine *et al.,* 1998)**

### 2.2.3 *Pathogenesis of E coli*

**Most *Escherichia coli* are harmless commensals which are part of the natural gastrointestinal flora in the lower intestine of warm-blooded animals. They are considered beneficial for maintaining a healthy intestinal ecosystem and have even been candidates for probiotic treatment to counteract a variety of enteric diseases. However, some subsets of *E. coli* have acquired specific virulence attributes that render them capable of causing a variety of illnesses in healthy humans and animals (Kaper *et al.*, 2004).**

Enterohemorrhagic *E coli* are characterized by the presence of Shiga toxin (Stx) genes, locus for enterocyte effacement (LEE), and a large molecular weight plasmid that encodes for a hemolysin. These 3 virulence factors are present in most *E coli* associated with bloody diarrhea and HUS in humans. The LEE is a large cluster of genes that collectively are responsible for the intimate attachment of the bacterium to the apical membrane of the enterocyte and subsequent destruction or effacement of the microvilli (Burland *et al.*,1998). The intimate attachment of the bacterial cell to the epithelium is attributed to the adhesin, intimin, and Tir, a bacterial protein, which is inserted into the host membrane and serves as the receptor for intimin. Both factors are part of LEE in

enteropathogenic *E coli* (EPEC) and EHEC. Intimin appears to be an essential component in initiating attachment, colonization, and the subsequent pathologic changes that follow infection with EPEC and EHEC (Dean Nystrom *et al.*, 1998). Outside of its role in the disease process in human infections, it is uncertain whether intimin has a role in the colonization of healthy cattle (Brown *et al.*,1997).

In addition to LEE, *E coli* O157:H7 possesses a large molecular weight plasmid that contains several putative virulence genes, including a pore-forming hemolysin (Pema *et al.,* 2001). Virulence plasmids are common features of pathogenic *E coli*, encoding toxins, adhesins, and other factors necessary for colonization, survival, and ability to cause disease in its animal host. The contribution of any of these plasmid-associated factors to disease is currently unknown.

*Escherichia coli* O157:H7 and other EHEC O serotypes produce a toxin similar in its amino acid sequence to *Shigella* toxin, hence the commonly used nomenclature for this *E coli* toxin in the literature, Shiga toxin or vero toxin. Among EHEC, there are 2 major immunologically distinct toxins, Stx1 and Stx2. Within the Stx2, there are additional antigenic variants. The Stx2v (variant)-producing *E coli* are associated with diseases in domestic animals, such as edema disease of swine. Enterohemorrhagic *E coli* that commonly cause human illnesses produce Stx1, Stx2, or both. The presence of the Stx2 in these EHEC has a profound influence on the progression of the disease from hemorrhagic colitis to HUS. As is common for many bacterial toxins, Stx consists of 2 subunits. The Stx-A subunit contains the enzymatic activity responsible for inhibiting protein synthesis, and the B-subunit acts as a lectin, binding the toxins to intestinal epithelial and kidney endothelial cells. The Stx is believed to be the major factor

contributing to the lesions in HUS, although the O157 lipopolysaccharide may also contribute to this disease syndrome (Karpman *et al.,* 1997). The virulence and pathogenesis of EHEC are associated with the presence of λ-like phages harboring these Stx genes.Deoxyribonucleic acid damaging agents activate the dormant, toxigenic bacteriophages of *E coli* O157:H7 from its latent state. The virus particle may be activated from its latent state in its *E coli* host as a response to signals present in the gastrointestinal tract (Acheson *et al.*,1998). Once released, this virus can infect susceptible *E coli* hosts, creating new toxin-producing cells. In addition to releasing the virion from its quiescent state in *E. coli,* DNA-damaging agents also can induce expression and release of toxin, consequences of which may be devastating to its animal host (Zhang *et al*.,2000). Therefore, antimicrobials that cause DNA damage in the bacterial cell (ie, quinolones) may be contraindicated for treatment of *E. coli* infection.

## 2.3 CLASSIFICATION OF *E. COLI*

*Enterotoxigenic E. coli (ETEC)*

Enterotoxigenic *E. coli* have adhesins in the form of fimbriae, which are species-specific (Karen, 1992). The fimbrial adhesins help them to adhere to specific receptors on enterocytes of the proximal small intestine (Todar, 2002). They produce enterotoxins referred to as the (heat-stable) toxin (Nataro and Kaper, 1998). The genes which are responsible for the encoding of the toxins may occur on the same or separate plasmids (Nataro and Kaper, 1998). The LT enterotoxin is very similar to cholera toxin in both structure and mode of action (Slutsker et al., 1997). The LT (heat-labile) enterotoxin binds to the same identical ganglioside receptors that are also recognized by the cholera toxin (GM1). The enzymatic activity is also said to be identical to that of the cholera

toxin (Nataro and Kaper, 1998). The disease requires colonization and elaboration of one or more enterotoxins, which are plasmid-encoded (Todar, 2002).

### 2.2.4 *Enteropathogenic E. coli (EPEC)*

**Enteropathogenic *E. coli* (EPEC) was the first group of *E. coli* strains recognized as pathogens following serological studies comparing strains cultured from outbreaks of neonatal diarrhoea with other strains isolated from healthy infants (Nataro and Kaper, 1998; Donnenberg and Whittam, 2001). Although such outbreaks are now rare in developed countries, this strain continues to be the leading cause of diarrhoea among infants from developing countries (Nataro and Kaper, 1998; Donnenberg and Whittam, 2001).**

**The pathogenesis of this group of *E. coli* has proved to be agreeable to genetic analysis, and several themes have emerged, reports Nataro and Kaper (1998). Enteropathogenic *E. coli* have an exacting pattern of adherence to tissue culture cells, which is allied with the presence of a large adherence factor (EAF) plasmid (Nataro and Kaper, 1998). Enteropathogenic induces watery diarrhoea similar to that of enterotoxigenic *E. coli* only that they do not possess the same colonization factors and do not produce *E. coli* ST or LT toxins (Michael *et al*., 1997). However, they produce a non-fimbrial adhesin designated intimin that mediates the final stages of adherence (Nataro and Kaper, 1998). Even though, EPEC do not produce LT or ST toxins, it has been reported that EPEC produce an enterotoxin similar to that of *Shigella*. This suggests that other virulence factors may be associated to those found in *Shigella* (Michael *et al.,* 1997; Nataro and Kaper, 1998).**

**In other studies, it has been established that adherence of EPEC to the intestinal mucosa is a complex process and may produce dramatic effects in the ultra-structure of the cells resulting in reorganization of actins in the vicinity of adherent bacteria (Donnenberg and Whittam, 2001). This process is called "attaching and effacing" of cells (Nataro and Kaper, 1998; Donnenberg and Whittam, 2001). Enteropathogenic *E. coli* are said to be "moderately-invasive" meaning they are not as invasive as *Shigella,* and unlike ETEC or Enteroaggregative *E. coli* (EAggEC), they cause an inflammatory response (Nataro and Kaper, 1998).**

**Diarrhoea and other symptoms of infections of these strains of *E. coli* probably are caused by bacterial invasion of host cells and interference with normal cellular signal transductions rather than by production of toxins (Donnenberg and Whittam, 2001). Some types of EPEC are referred to as Enteroadherent *E. coli* (EAEC) or diffuse adherent *E. coli* (DAEC) based on specific patterns of adherence (Michael *et al*., 1997, Donnenberg and Whittam, 2001). Enteropathogenic *E. coli* have been documented as important causes of traveller's diarrhoea in Mexico and in North Africa (Michael *et al*., 1997; Nataro and Kaper; 1998). This group of *E. coli* has been reported in HIV/AIDS patients with chronic diarrhoea in Bangui Central Republic of Africa (Mossoro *et al*., 2002).**

### 2.2.5 *Enteroaggregative E. coli (EAggEC)*

**The distinguishing features of these strains are their ability to attach to tissue culture cells in an aggregative manner (Michael *et al.,* 1997). These strains are associated with persistent diarrhoea in young children (Michael *et al.,* 1997, Nataro and Kaper, 1998). They resemble enterotoxigenic *E. coli* (ETEC) strains in that they adhere to the intestinal mucosa and cause non-bloody diarrhoea without invading or causing inflammation (Michael *et al.,* 1997). This in itself suggests that the organisms produce a toxin of some sort.**

**A distinctive heat-labile plasmid-encoded toxin called the EnteroAggregative ST (EAST) toxin which has been isolated from these strains.Enteroaggregative *E. coli* also produce a haemolysin related to the haemolysin produced by *E. coli* strains involved in urinary tract infections. However, the roles of the EnteroAggregative ST (EAST) toxin and the haemolysin gene in virulence have not been established (Nataro and Kaper, 1998). The significance of EAggEC strains in human disease is controversial though they are also armed with a thin fimbrae, which is approximately 2 to 4 nm that they use to bind tissue matrix proteins.**

### 2.2.6 *Enterohemorrhagic E. coli (EHEC)*

**Enterohemorrhagic *E. coli* are considered "moderately invasive" though nothing is known concerning their colonization antigens, however, fimbriae are presumed to be involved. The bacteria do not invade mucosal cells as readily as *Shigella, Yersinia* or *Aeromonas,* which are armed with fimbrae (Nataro and Kaper, 1998). Nevertheless, infection of human intestinal epithelial cells by these bacteria is said to activate the enzyme mitogen activated protein (MAP) kinase (p38 and ERK 1/2) and NFk B signaling pathways, both of which are required for the induction of an intestinal epithelial cell pro-inflammatory response (Byrne *et al.,* 2002).**

**Enterohemorrhagic *E. coli* (EHEC) are represented by a single serotype of *E. coli* called *E. coli* O157:H7, which causes diarrhoeal set of symptoms discrete from that of enteroinvasive *E. coli* (EIEC) and *Shigella* in that there is copious bloody discharge without fever (Michael *et al.,* 1997). *Escherichia coli* O157:H7 is a gram-negative rod-shaped bacterium. The letter "O" in the name refers to the somatic antigen number, whereas the "H" refers to the flagella antigen (Nataro and Kaper, 1998). *Escherichia coli* O157:H7 serotypes apparently arose because of horizontal gene transfer of virulence factors (Whitam, 1988).**

**According to Kassenborg *et al.* (2000) and other authors (Prier *et al.,* 2000; Gansheroff and O'Brien, 2000; Donnenberg *et al.*, 2001; Todar, 2002) these *E. coli* strains have been recognized as a cause of serious diseases often linked to eating of inadequately cooked hamburger meat. Pediatric diarrhoea caused by these strains can be fatal due to acute kidney failure (Galane and Le Roux, 2001).**

**The permutation of letters and numbers in the name of *E.coli* O157:H7 refers to the specific markers found on these bacteria's cell surface; these letters and numbers tell between the dangerous O157:H7 variety from other types of *E. coli*. The virulence of *E. coli* O157:H7 is due to its ability to produce Shiga-like toxins, or verotoxins (Wells *et al*., 1991). Shiga-like toxins hinder protein synthesis in eukaryotic cells and play a role in hemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS). The toxin is also said to accelerate this by causing damage to endothelial cells in the kidneys, pancrease, brain, and other organs, thus inhibiting the ability of these organs to function (Donnenberg and Whittam, 2001).**

## **2.3** SEROPATHOTYPES

**VTEC is considered a natural inhabitant of ruminants and is very frequently isolated from these animals. However, only a small subset of VTEC from ruminants should be considered as potential human pathogens, as VT alone is not sufficient to induce human disease (Law, 2000).**

**More than 400 different serotypes of VTEC have been isolated from humans but only few are associated with the majority of human EHEC cases (Scheutz & Strockbine, 2005). A classification system based on the concept of "seropathotypes" has been compiled by Karmali and colleagues (Karmali *et al.*, 2003). This system classifies VTEC serotypes according to their ability to induce disease. The serotypes are ranked in five groups (A-E) ranging from the most pathogenic serotypes (A) to those that have never been associated with human disease (E). This classification is based on the reported occurrence of different serotypes in human outbreaks and by the frequency with which they are reported to have induced haemolytic uraemic syndrome (HUS).**

**The panel of the European Food Safety Authority (EFSA) concerned with Biological Hazards has recommended that animal and foodstuffs monitoring in the EU should initially be concentrated on VTEC O157, since this serotype is most frequently associated with severe human infections and HUS, but that monitoring should be extended to other serotypes such as O26, O103, O91, O145 and O111 that, after O157, are the serotypes most frequently causing human infections in Europe (Anonymous, 2007).**

### 2.3.1 *VTEC O157 (Seropathotype A)*

**VTEC O157:H7 is the prototype bacterium for EHEC and is the serotype most frequently isolated from outbreaks and severe human disease worldwide (Karmali *et al.*, 2003).**

## 2.4 NON-SORBITOL AND SORBITOL-FERMENTING VTEC O157

**In contrast to other *E. coli* strains, VTEC O157:H7 strains cannot rapidly ferment sorbitol, do not produce ß-glucuronidase and are generally, resistant to the antimicrobial agent tellurite. These are all features that can be used to identify VTEC O157:H7 strains. Due to their inability to rapidly ferment sorbitol, VTEC O157:H7 are often referred to as non-sorbitol fermenting (NSF) *E. coli* O157. However, some strains can ferment sorbitol rapidly within 24 h of incubation and these are referred to as sorbitol fermenting (SF) *E. coli* O157 (Karch & Bielaszewska, 2001).**

**The SF VTEC O157:H- strains are non-motile due to a "12-p" deletion in the fliC gene (Monday *et al.*, 2004). They produce ß-glucuronidase and carry vtx2 as their sole VT gene (Ammon *et al.,* 1999). Furthermore, they are not tellurite-resistant, as they lack the "tellurite adherence conferring island" (TAI) present in NSF VTEC O157:H7 (Tarr *et al.,* 2000). Moreover, SF VTEC O157: H- has acquired specific adhesion factors, novel pili, encoded by spf that distinguishes them from other *E. coli* (Friedrich *et al.,* 2004).**

## 2.5 THE GENOME OF VTEC O157:H7

**Genome comparison studies have revealed a high degree of genomic diversity within the VTEC O157 population, which is attributed to many events of insertion/deletion and recombination of DNA. Most of these events seem to be prophage mediated or driven by other mechanisms of horizontal gene transfer (Ohnishi *et al.*, 2002). Most VTEC O157:H7 strains carry a ~90 kb large plasmid, pO157 (often also referred to as the 60 MD plasmid). A similar but not identical plasmid, pSFO157, is found in SF VTEC O157:H- (Friedrich *et al.,* 2004).**

**The first two strains of VTEC O157:H7 that were whole genome Sequenced were "EDL 933" (Perna *et al*., 2001) and "Sakai O157" (Hayashi *et al.,* 2001). When all sequence data were compiled they revealed that both strains had a genome of ~5.5 Mb which was ~0.9 Mb larger than the earlier sequenced genome of the non-pathogenic K12 laboratory strain, "MG 1655". Apart from a common, highly conserved backbone of 4.1 Mb (shared by all three strains), the VTEC O157:H7 strains had about 1.34 million additional base pairs that not could be found in K12. On the other hand, about half a million of the base pairs from the K12 genome were missing in both sequenced VTEC O157:H7 strains. The additional "VTEC O157:H7-specific" 1.34 Mb were organized into several hundred "gene cassettes", O-islands or S-loops varying in size from 19 bp up to more than 15 kb. Prophage and prophage-like elements were abundant in these gene cassettes and about two-thirds of the Sakai O157 genome consisted of such elements (Ohnishi *et al.,* 2002).**

### 2.5.1 Evolution of VTEC O157

**In 1993, Whittam and colleagues suggested that *E. coli* O55:H7, an EPEC Serotype known to cause infantile diarrhea, could be the ancestor of VTEC O157:H7 (Whittam *et al.,* 1993). Feng and colleagues elaborated on this hypothesis and in 1998 proposed a stepwise evolution model for VTEC O157:H7 with O55:H7 as its progenitor (Feng *et al.,* 2000). Another study based on differences in single nucleotide polymorphisms (SNPs), located in the stable part of the genome, takes this evolution model even further.(Leopold *et al.,* 2009). In that study the authors even estimated the time spans in which the different subtypes evolved. For instance the SF O157:H- strain, commonly found among HUS cases in Germany is believed to have emerged 7000 years ago and Cluster 1 of Subgroup C strains, found among humans e.g. the" American spinach outbreak strain" (Manning *et al.*, 2008) might have emerged 3,000 years ago.**

### 2.5.2 VTEC O157:H7 in a historical perspective

**As early as from the 1960s, publications from Argentina have described HUS cases in children preceded by a period of diarrhea In an article published in 1972, Gianantonio and colleagues reviewed 678 Argentinean HUS cases in children from 1957 to 1972 where no etiological agent could be identified (Gianantonio *et al.,* 1973).**

**VTEC was first described in Canada in the late 1970s by Konowalchuk and colleagues. In 1982 it was first demonstrated that *E. coli* strains could produce a "shiga like toxin" (O'Brien *et al.,* 1982).**

**A year later, O'Brien and Laveck managed to purify verotoxin from an *E.coli* strain, concluding that the toxin was both structurally and antigenically similar to the Shiga toxin produced by *Shigella dysenteriae* type 1, and therefore described the new toxin as a "shiga like toxin" (O'Brien *et al*., 1983).**

**In 1982 Riley and colleagues were able to link a multistate food-born outbreak in USA (involving hamburger patties) to patients with bloody diarrhoea (HC). A rare serotype "*E. coli* O157:H7" was isolated from stool samples and the strain was later shown to produce VT (Riley *et al.,* 1983).**

**Karmali and colleagues established that HUS could be caused by VTEC O157:H7 and VTEC of other serotypes, following the observation that, production of VT was linked to HUS, it could also be confirmed that HUS cases among children in Argentina were caused by VTEC (Karmali *et al.,* 2009).**

### 2.5.3 Virulence factors of VTEC O157 in humans

#### *2.5.3.1* ***Verotoxin (VT)***

**Verotoxins are the main virulence factor of EHEC. The name derives from their specific cytotoxicity to Vero cells (African green monkey kidney cells).VTs belong to a family of AB5 toxins, characterized by a single enzymatically active A subunit ~32kDa linked to a pentamer of five identical receptor-binding B subunits, each ~7.7kDa. Based on toxin-neutralization and nucleotide sequence analyses the verotoxins are classified in two major groups, VT1 and VT2, showing approximately 60% nucleotide sequence identity. These two major groups can be further divided into several variants. VTEC strains may harbor a single VT alone or possess several different (or similar) variants in combinations (reviewed by Müthing *et al*., 2009).**

**Generally, VT2 has been more closely associated with severe disease and HUS, than has VT1 (Jenkins *et al.,* 2003). VT2 has also proved to be 1000 times more potent as an agent toxic to human renal endothelial cells, than VT1 (Louise & Obrig, 1995).VTs are encoded by a number of heterogeneous temperate lambdoid phages that follow the lysogenic pathway and are inserted as prophages at specific insertion sites in the bacterial genome (reviewed by Allison, 2007). VTEC O157:H7 can carry several different phages and at least five different insertion sites have been described, whereof yehV and wrbA are those most commonly occupied (Serra-Moreno *et al.,* 2007).Events that provoke the bacterial SOS response, such as exposure to UV light or antibiotics, lead to induction of the phage's lytic cycle. The prophage is then excised and the bacterium starts to produce numerous phage particles and VT. This is followed by cell lysis whereby the phage particles and VT are released (Zhang *et al*., 2000). The phage particles released can also transduce other phage-sensitive bacteria in the gut, thus increasing the number of bacteria producing VT (Gamage *et al.,* 2003).**

**Furthermore, excision of integrated phages can take place without lysis of bacterial cells. For instance HUS patients can shed non-VTEC strains identical to the disease-causing EHEC strains, the only difference being absence of VT (Bielaszewska *et al*., 2007). It has also been demonstrated that identical VT-phages can be present at different insertion sites in strains from human outbreaks, indicating that the phages can move between different insertion sites (Bielaszewska *et al.,* 2006b).**

**The pentameric B unit of the VTs binds specifically to glycolipid receptors, Gb3 (globotriaosylceramide) or Gb4 (globotetraosylceramide), that are expressed on the cell surface by a variety of epithelial and endothelial cells (Gb4 is preferred by subgroup VT2e whereas all other VTs prefer Gb3 (Lingwood *et al.,* 1998). After attachment the toxin is 1`internalized into the cell by receptor-mediated endocytosis (Römer *et al.,* 2007). The toxin-receptor complex is transported via the trans-Golgi network to the endoplasmatic reticulum (ER) (Sandvig *et al*., 1992). Near the nuclear membrane the ~32kDa Subunit A is cleaved by a protease, furin, into a catalytically active A1 fragment and an A2 fragment (Garred *et al.,* 1995). The A1 fragment is released into the cytosol where it exerts tRNA Nglycosidase activity that removes an adenine moiety from 28S rRNA in the eukaryotic 60S ribosomal subunit. This modification inhibits protein synthesis and leads to cell death, as the acceptor site for aminoacyl-tRNA is blocked. There are also VT-resistant cells, in which VT is engulfed and rendered ineffective by lysosomes (Hoey *et al*., 2003).**

**In addition to the cytotoxic effect, VT can induce apoptosis, programmed cell death, characterized by DNA fragmentation, cell shrinkage, membrane blebbing and condensation of nuclear chromatin. This is mediated via an independent pathway regulated by proteins from the BcL-2 family (Jones *et al.,* 2000; Suzuki *et al.,* 2000).**

**It has also been shown in renal carcinoma-derived cells that the specific binding of the B subunit of VT1 to Gb3 in itself produces intracellular signals which remodel cytoskeletal organizing proteins (Takenouchi *et al.,* 2004).**

**Furthermore, VTs stimulate epithelial cells and a variety of non-endothelial cells to secrete inflammatory mediators (cytokines, chemokines) that induce a pro inflammatory response exaberating the detrimental effects of VTs on endothelial cells (reviewed by Naylor *et al.,* 2005a).**

### 2.5.4 Variance in virulence among VTEC O157 strains

**Based on genomic differences identified by octamer-based genome scanning (OBGS) (Kim *et al*., 1999), lineage-specific polymorphism assay.(LSPA) (Yang *et al*., 2004) and micro array based comparative genomic hybridization (mCGH) (Zhang *et al*., 2007), VTEC O157:H7 strains have been categorized in three lineages. Lineage I comprises strains commonly isolated from both cattle and humans, lineage II comprises strains isolated primarily from cattle whereas the third lineage, I/II, has not been strictly characterized regarding host distribution. The fact that lineage II strains are seldom isolated from humans implies that these strains are less virulent for humans or ineffectively transmitted to humans from bovine sources (Kim *et al*., 1999). Furthermore, it has been shown that strains from lineage I and lineage I/II produce significantly more VT2 than strains from lineage II and that this is due to genetic differences in the prophages carrying the VT2 genes from the different lineages (Zhang *et al*., 2009).**

**A study in Silico comprising six different DNA typing methods (Laing *et al.*, 2009) reported that lineage I/II strains belonged to a putative hyper-virulent clade of strains, called clade 8 (Manning *et al.,* 2008). By developing a single-nucleotide polymorphism (SNP) typing system, Manning and colleagues were able to distribute 519 VTEC O157:H7 strains into nine evolutionary clades. Using clinical data from isolated strains they found that patients infected with VTEC O157:H7 strains from one of the clades, clade 8, were seven times more likely to develop severe disease. Moreover, these patients were more likely to be younger (ages 0-18) than patients infected with strains from the other clades. Clinical outbreak data also revealed that clade 8 strains led to remarkably high rates of hospitalization (average 63%) and HUS (average 13%) compared with other outbreak strains.**

### 2.5.5 **VTEC O157 in humans**

#### *2.5.5.1* ***Disease in humans***

**Clinical manifestations of VTEC O157:H7 vary from no symptoms at all (asymptomatic carriers), to mild watery diarrhea, bloody diarrhea, to severe complications such as haemolytic uraemic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) and even death (reviewed by Mead & Griffin, 1998). The infectious dose of VTEC O157:H7 is very low, estimated to be <50 (Tilden *et al.,* 1996) or more, a few hundred bacteria (Bell *et al*., 1994). After a mean incubation period of 3 days (can vary from 1 to 8 days) infected humans develop watery diarrhea, vomiting (30-60% of cases) and abdominal pain with cramps. About 30% of patients have mild fever, usually observed in the early stages of the disease (reviewed by Mead & Griffin, 1998). About 1-3 days after onset, over 70% of the patients develop bloody diarrhea (hemorrhagic colitis, HC) though lower frequencies have also been reported. The amount of blood in faeces varies from traces to almost entirely blood (reviewed by Tarr *et al.,* 2005).**

**Most patients recover spontaneously within a week of onset, whereas a small subset of cases progress to HUS or other complications. The proportion of patients who progress to severe bloody diarrhea and/or HUS varies for different strains of VTEC O157, as well as age and immunological status of the infected patient. In sporadic cases, 3-7% of cases progress into HUS whereas in specific outbreaks a HUS incidence of up to 20% has been reported (reviewed by Mead & Griffin, 1998). The risk that a child <10 years old with a diagnosed VTEC O157:H7 infection will develop HUS is estimated to be about 15% (reviewed by Tarr *et al.,* 2005). HUS occurs 5-13 days after the onset of diarrhea and is characterized by haemolytic anaemia with fragmented erythrocytes, thrombocytopenia and acute renal failure (reviewed by Karch *et al.,* 2005). The syndrome can be life threatening and patients need to be hospitalized and given intensive care including intravenous fluid perfusions. About half of all HUS patients require dialysis, 80% need erythrocyte transfusions, while 3-5% succumb (reviewed by Tarr *et al*., 2005).**

**Elderly persons also run a greater risk of developing HUS or a condition resembling HUS, called TTP. This is more common in adults and, in addition to acute renal failure, is also characterized by fever and neurological symptoms such as lethargy, severe headache, convulsions and coma (reviewed by Paton & Paton, 1998). Other acute complications reported after VTEC O157:H7 infections are cardiac dysfunction, pancreatitis, stroke, rectal prolapse and colonic perforation with peritonitis (reviewed by Karch *et al.,* 2005).A small subset of patients develop chronic renal sequelae or other sequelae such as diabetes mellitus, neurological disorders, hypertension and colonic strictures (reviewed by Karch *et al*., 2005).**

### 2.5.5.2 Pathophysiology in humans

**After ingestion, acid-resistance mechanisms of VTEC O157:H7 facilitates their survival through the low pH of the stomach (Palermo *et al.,* 2009). The bacteria become attached by adhesins to the epithelium in the colon (reviewed by Spears *et al*., 2006). Quorum sensing (QS) mechanisms.**

**Probably regulate expression of the bacterial genes needed for attachment, so that even small quantities of bacteria succeed in attaching (Pacheco & Sperandio, 2009). The bacteria colonize enterocytes via the TTSS pathway and A/E lesions are created (reviewed by Spears *et al.,* 2006).**

**VT and endotoxin (LPS) are released and absorbed across the gut epithelium. VT, LPS and possibly other virulence factors lead to an increase in pro-inflammatory cytokines from host cells and subsequent release of chemokines from inflammatory cells. The susceptibility for VT in the intestine microvascular endothelial cells is exacerbated by this pro-inflammatory response (reviewed by Palermo *et al*., 2009). VT and LPS activate thrombocytes, and the endothelial injuries, activation of the coagulation cascade and inhibition of fibrinolysis lead to formation of thrombi that occlude capillaries and small arterioles. The intestinal vascular injury leads to ischaemia and necrosis that, together with the inflammatory response, probably cause the bloody diarrhea (reviewed by Tarr *et al*., 2005).**

**VT and LPS can also enter the bloodstream bound to polymorphonuclear leukocytes (VT) (Brigotti *et al.,* 2008) and thrombocytes (LPS), (Ståhl *et al.,* 2006) they can reach the kidneys. In the kidneys, Gb3 receptors are expressed on endothelial glomerular cells, mesangial and tubular epithelial cells. The pro-inflammatory response and actions of VT elicit a pronounced endothelial swelling and the pro-thrombotic mechanism leads to occlusion of small blood vessels in the glomeruli. By acting on renal tubular cells, VT induces apoptosis. When acting in combination, these mechanisms lead to thrombocytopenia and acute renal failure (reviewed by Tarr *et al.,* 2005). The anaemia with fragmented erythrocytes, as seen in HUS, is caused by mechanical breakdown when the erythrocytes pass partly occluded blood vessels, and/or by oxidative damage (reviewed by Proulx *et al*., 2001).**

**Another organ containing endothelial cells with Gb3 receptors are the brain where the VTs can cause endothelial damage and thrombotic disorders leading to neurological symptoms (reviewed by Proulx *et al.,* 2001).**

### 2.5.6 Routes of transmission to humans

**In general, the different routes of transmission of VTEC O157:H7 to humans are via food, water or various items either directly or indirectly contaminated by ruminant faeces. As the infectious dose is very low the bacterium's excellent capacity to survive over time in different environments is of decisive importance.**

#### *2.5.6.1 Drinking and recreational waters*

**There is a complex interaction of environmental and behavioral factors of both animals and humans, which facilitate the spread of *E. coli* O157:H7 infections. Poor sanitation and hygiene conditions as well as lack of or little environmental awareness among people is considered as a major cause of source water contamination (Zamxaka *et al.,* 2004; Phaswana-Mafuya, 2006). Example of such ways is agricultural practices that involve usage of sewage waters and/or cattle manure on farms. Another practice is uncontrolled waste disposal, bathing and swimming in water sources such as rivers and dams, which serve as sources to municipal water supplies, answering calls of nature in fields (Defecation) and grazing of cattle next to catchment areas (Phaswana- Mafuya, 2006; WHO, 2006a).**

*2.5.6.2* ***Milk and milk products***

**The contamination of milk by *E. coli* O157:H7 is often suspected to occur during the milking process. Due to contamination of milk during its collection and/or processing, milk and milk products has been implicated in *E. coli* O157:H7 disease outbreaks. The bacteria have been isolated from cheese sandwiches (Kassenborg *et al.,* 2004). This has been possible because the organism is acid tolerant and can grow under low acidic conditions (Conner and Kotrola, 1995).**

### *2.5.6.3 Fruits, vegetables and their products*

**The common type of fruits and vegetables that have always been implicated in *E. coli* O157:H7 outbreaks are fresh and ready to eat cut fruits and vegetables like tomatoes, lettuce, cucumber, onions and carrots, especially those used in salads (Mukherjee *et al*., 2004). There have also been numerous outbreaks of *E. coli* O157:H7 implicating apple juice (Cody *et al.,* 1999). Most researchers have established that the commonest cause of *E. coli* O157:H7 on vegetables is the use of cattle manure or irrigation waters that are potentially contaminated with *E. coli* O157:H7 in the growing of fruits and vegetables (Johannessen *et al.,* 2005). Outbreaks of diseases caused by shiga toxigenic *Escherichia coli* have been associated with the consumption of leafy lettuce, potatoes, radish sprouts and alfalfa sprouts (Barrett *et al*., 2001).**

**The rapid spread of *E. coli* O157:H7 infection due to the consumption of contaminated vegetable is attributed to the increase in microbial numbers during the process of cutting and slicing of the vegetables (Cassin *et al*., 1998). The process of cutting and slicing, somehow, blow up the bacterial numbers to the tune of six and seven fold. The cases of *E. coli* O157:H7 on vegetables have been common mostly in salad bars (Brooks *et al.,* 2004).**

*2.5.6.4* ***Food***

**Contaminated food of bovine or bovine origin such as unprocessed meat, undercooked hamburgers, cold fermented sausages, unpasteurized milk, yoghurt and cheese made from unpasteurized milk are common causes of VTEC O157:H7 infections. Vegetables and fruit products e.g. unpasteurized apple juice, and also vegetables and salad ingredients such as lettuce, spinach, alfalfa and radish sprouts, have also been associated with several outbreaks. These food products may have been contaminated by direct spreading of cattle manure on growing crops or indirectly via contaminated irrigation or processing water (Hussein & Sakuma, 2005).**

**The largest known VTEC O157:H7 outbreak to date was caused by radish sprouts in Sakai City, Japan in 1996, where 6,000 mostly School children became ill and 1.2% of them developed HUS (Michino *et al.,* 1999). In 1996, 522 people in Scotland were infected and 22 died after eating contaminated meat from a butcher's shop (Cowden *et al.,* 2001).**

**Water in private wells contaminated by grazing cattle is a common infection route of VTEC O157:H7 in Scotland (Strachan *et al*., 2006). In an outbreak in Walkerton, Ontario, Canada in 2000, 2,300 people became ill, 28 developed HUS and 7 died after drinking municipal water contaminated with both *Campylobacter jejuni* and VTEC O157:H7 (Richards, 2005).**

**In autumn 2002 there was a Swedish VTEC O157:H7 outbreak in the province of Skåne affecting 30 patients all of whom had consumed infected cold fermented sausage. Of these, 13 (43%) were hospitalized and 9 (30%) developed HUS (Sartz *et al.,* 2007). Another outbreak occurred in southwest Sweden in the summer of 2005 where 135 cases, including 11 (8%) HUS patients, proved culture-positive for VTEC O157:H7 after eating contaminated fresh lettuce (Söderström *et al*., 2008).**

*2.5.6.5 Direct or indirect contact with animals*

There are numerous reports of cases where people have contracted an EHEC infection after visiting a farm where ruminants have been found shedding VTEC O157:H7. Often these farms are so-called "visiting farms" where children are allowed to meet and pet animals (Strachan *et al*., 2006). Non-ruminant animals on these farms may also be transmitting VTEC O157:H7 infection after they have picked up the bacterium from ruminants (Pritchard *et al.*, 2001).

*2.5.6.6 Exposure from environment*

Ruminants especially cattle can excrete high levels of VTEC O157:H7 (>105 cfu/g) in feaces (Ogden *et al*., 2004). The bacterium spreads to the environment via grazing ruminants or by direct spreading of infected manure on land. After heavy rainfall the bacterium can spread further in the environment by overland runoff.

VTEC O157:H7 has a great capacity to survive for lengthy periods in manure, soil and water. Survival in bovine faeces ranges from 46-126 days (Fukushima *et al*., 1999) and up to 90 days in cattle slurry (McGee *et al*., 2001). In water the bacterium can survive for 40 days at 21°C and for > 70 days at 5°C (reviewed by Fremaux *et al*., 2008). In studies on manure amended soil, VTEC O157:H7 persisted for 25 to as long as 231 days, depending on experimental environmental conditions and inoculum levels (reviewed by Fremaux *et al*., 2008).

*2.5.6.7 Person to person infections*

**The very low infectious dose facilitates spreading of VTEC O157:H7 infection from person to person via the faecal–oral route. Deficient hygiene after toilet visits enhances this transmission route which is common among family members and institutional settings such as day-care centers and homes for the elderly. In outbreaks, several routes of transmission are often involved e.g. in food-borne outbreaks person to person spread is common between patients who have eaten infected food.**

## 2.6 EPIDEMIOLOGY OF INFECTION IN HUMANS

**VTEC infections show a seasonal variation, with an increase in human cases during summer and early autumn (PEN, 2006a). The incidence peaks in children ≤ 4 years, but falls rapidly with increasing age (PEN, 2006a). Severe complications are more commonly seen in children (HUS) and elderly patients (Both HUS and TTP) (Karmali *et al.,* 2009) whereas asymptomatic carriers are more common in the age groups in-between.**

**Most cases are sporadic, although large outbreaks do occur (Rangel *et al.,* 2005). Reported incidences of human VTEC infections and VTEC serotypes vary between different parts of the world. VTEC O157:H7 is the predominant serotype in North America and Japan (Griffin *et al.,* 2009; Watanabe *et al.,* 2009). Argentina has the highest global incidence rate of HUS in children ≤ 5 years old (15 per 100,000), the predominant etiological agent being VTEC O157:H7 (Rivas, 2009).**

## **2.7** VTEC O157:H7 IN ANIMALS

**Cattle constitute the main reservoir for VTEC O157:H7, it has also been isolated from sheeps, goats and water buffalos (Pritchard *et al.,* 2000). Wild ruminants can also act as a potential reservoir; for instance VTEC O157:H7 has considered a significant source of infection in humans, being isolated from wild deer (Renter *et al.,* 2001). Occasionally, the bacterium has been isolated from non-ruminant species e.g. horse, dog, rabbit, seagull, starling, wild boar and rat (Jay *et al.,* 2007). These animals are not considered as hosts, but rather as vectors transiently colonized by the bacterium following contact with ruminant faeces (reviewed by Caprioli *et al.,* 2005).**

## **2.8** FACTORS AFFECTING GROWTH OF *E. COLI* O157:H7

**Like all other bacteria the survival and growth of *E. coli* O157:H7 depend on the interaction between intrinsic and extrinsic factors (Buchanan and Michael, 1997). These factors include temperature, pH, water activity, salinity, nutrients and background micro flora (Buchanan and Michael, 1997). These factors are discussed in this section.**

### **2.9.*1Temperature***

**Many *E. coli* O157:H7 isolates do not grow well, if at all, at temperatures above 51°C . These bacteria are differentiated from other Enterobacteriaceae based on their ability to grow and produce gas in *E. coli* broth at 44 °C (Buchanan and Michael, 1997). The optimal temperature for growth of *E. coli* O157:H7 is approximately 37°C (Buchanan and Michael, 1997). However, the upper temperature for *E. coli* O157:H7 growth is culture medium dependent (Buchanan and Michael, 1997).**

**Most strains of *E. coli* O157:H7 grow in Brain Heart Infusion (BHI) broth at 45 °C, as opposed to *E. coli* broth where it optimally grows at 44 °C (Buchanan and Michael, 1997). The minimum growth temperature for *E. coli* O157:H7 under optimal conditions is approximately 8– 10°C. However, the organism can survive refrigeration temperature but show a decline in concentration (Buchanan and Michael, 1997).**

***2.9.2 pH***

**The pH of water and food products influences the survival of microorganisms (Presser *et al.*, 1997). The pKa of an acid and the pH of water or food determine the amount of dissociated and undissociated acid that will occur in the water or food product (Abdulraouf, 1993; Presser *et al.,* 1997).**

**High levels of undissociated acid in water or food; leads to the acid being able to permeate the cell wall of the bacteria and become dissociated as a result of higher intracellular pH (Barbut and Griffiths, 2003). *Escherichia coli* O157:H7 has been reported by various researchers to grow at low pH values (Samelis *et al.,* 2005). This property is believed to be under the control of rpoS– regulated proteins, which are activated when, *E. coli* O157:H7 cells are subjected to low pH (Leyer *et al*., 1995; Cheville *et al.,* 1996).**

**Low pH is also believed to induce an increase in saturated fatty acids like palmitic acid and reduces the concentration of unsaturated fatty acids such as cis–vaccenic acid in the bacterial cell membranes (Samelis *et al.,* 2005). However, this property is said to be dependent with other growth factors such as temperature and water activity (Aw) and that *E. coli* O157:H7 cells grown at temperatures equal to or less than 4 $^{o}$C tend to be more acid resistant than cells grown at temperatures above 15$^{o}$C (Samelis *et al.,* 2005). Acid tolerance in *E. coli* is a complex phenomenon (Buchanan and Michael, 1997). *Escherichia coli* cells in the stationary phase of growth are substantially more acid tolerant than cells in the exponential phase. This increased tolerance is associated with expression of genes regulated by the rpoS sigma factor operon (Buchanan and Michael, 1997).**

### *2.9.2 Water activity (Aw)*

**The intrinsic factors of a food product like water activity are very important in determining the product's shelf life and safety with respect to microbial quality. A reduced Aw affects bacterial cells by increasing the lag phase and decreasing the growth rate, resulting in a reduction in bacterial population (Barbut and Griffiths, 2003). The effect of water activity on the survival and growth of *E. coli* O157:H7 focuses primarily on the effect of humectants (Buchanan and Michael, 1997). *Escherichia coli* O157:H7 can survive for many weeks when desiccated, particularly at refrigeration temperatures. Under ideal conditions, the minimum water activity for its growth is approximated at 0.98 (Buchanan and Michael, 1997).**

### *2.9.3 Nutrients*

**New selective media have been developed to increase the effectiveness of *E. coli* O157:H7 isolation (Malihe and Kadir, 2000). The agar medium mostly used for the isolation of *E. coli* O157:H7 is Sorbitol MacConkey Agar (SMAC agar) (Jennifer *et al.,* 1998).**

**This medium contains 1% sorbitol in place of lactose in the standard MacConkey medium. Sorbitol-MacConkey agar plates are inoculated with the suspected *E. coli* O157:H7 positive specimen and examined after 18 to 24hours of incubation at 37$^{o}$C for the presence of colourless, sorbitol-negative colonies (Jennifer *et al.,* 1998). A few non sorbitol fermenting colonies may be selected for testing as potential *E. coli* O157:H7 (Malihe and Kadir, 2000).**

### *2.9.4 Background micro flora*

**High levels of competing background micro flora always compound the difficulty of isolating *E. coli* O157:H7 (Ogden *et al.,* 2000). In a report by Stewart (1999), the survival of verocytotoxic *E. coli* serotype O157 in cattle dosed with commensal *E. coli* and *Proteus mirabilis* strains recovered from ruminant faeces was highly reduced ( Bell *et al*.1999) also reported that *Pseudomonas aeruginosa* from the rumen of sheep inhibited the growth of *E. coli* O157 in vitro. In their study, these authors argued that *P. aeruginosa* strains from different animals produced different bacteriocins (pyocins), which they suspected had an ecological role in determining the dominant biotype (Bell *et al*., 1999; Stewart, 1999).**

**Two pigments from *P. aeruginosa* (pyocyanin and fluorescin) have also been shown to inhibit both commensal and *E. coli* serotype O157- strains (Stewart, 1999). It seems that some of the bacteria interacting directly with *E. coli* O157:H7 in the ruminant gut are relatively minor members of the gut micro biota (Stewart, 1999). Such bacteria may also influence the survival of *E. coli* in shed faeces and in the soil.**

**Bell (1999) and Stewart (1999) suggested that pyocyanin is presumably active if present in media exposed to air. In a separate experimental study on the effect of competitor micro flora on the growth kinetics of *E. coli* O157:H7; Duffy *et al.,* (1999) noted that growing *E. coli* O157:H7 together with *Hafnia alvei* in the same growth media; lengthened lag phase for *E. coli* O157:H7. However, *H. alvei* had a much shorter lag phase; suggesting that *H. alvei* inhibited *E. coli* O157:H7 under similar growth conditions.**

### *2.9.5 Salinity*

Various researchers have reported the use of salts in foods as food preservative agents (Buchanan and Michael, 1997). Sodium benzoate used together with fumaric acid has served well in the preservation of apple cider against spoilage by *E. coli* O157:H7 (Buchanan and Michael, 1997). When this preservative mixture was added to apple cider, Buchana and Michael (1997) observed a 5 log10 reduction in *E. coli* O157:H7 population. The above treatment was also found to be very efficient by Chikthimmah *et al.*,(2003) when the holding temperature of cider juice was increased from 25 to 35 $^{o}$C. At 35 $^{o}$C, no *E. coli* O157:H7 cells were recovered from the juice. Callaway *et al.*, (2003) equally established that water supplemented with Sodium chlorate reduced *E. coli* O157:H7 population in the faecal shedding of test cattle.

### *2.9.6 Irradiation*

Food safety can also be improved by food irradiation, a process whereby energy is applied to the food from a radioactive source (cobalt or cesium), electron beams, or x-rays (Lagunas-Solar, 1995). The chemical changes thus produced in the food impair the ability of microorganisms to grow. The use of ionizing irradiation has been realized to eliminate or reduce contamination of foods by pathogens, such as *E. coli* O157:H7.

Foods that are treated with low-level gamma radiation shows reduced levels of pathogenic *E. coli* O157:H7 (Zafer *et al.,* 2007). This technique works better when used in combination with refrigeration to inactivate *E. coli* O157:H7 in foods (Buchanan *et al.,* 1998). Buchanan *et al.,* (1998) equally demonstrated that low dose irradiation could readily eliminate *E. coli* O157:H7 from fresh apple juice while maintaining the product at refrigeration temperatures (Buchanan *et al.,* 1998). Regrettably, Buchanan *et al*. (1998) reported that mutant strains of *E. coli* O157:H7 could occur in foods and this could jeopardize the efficacy of radiation. However, Thayer and Boyd (1993) concluded in their study that application of irradiation energy at doses ranging between 1.5 and 3.0 kGy at low temperatures was sufficient to inactivate *E. coli* O157:H7 cells.

## 2.10 DIRECT IMPACT OF WATER AND FOOD CONTAMINATED WITH *ESCHERICHIA COLI* O157:H7 ON PUBLIC HEALTH

### 2.10.1 Haemolytic uremic syndrome (HUS) and Hemorraghic colitis (HC)

The most serious complication of *E. coli* O157:H7 infection is haemolytic-uremic syndrome (HUS). This is a potentially life-threatening condition characterized by haemolytic anaemia, thrombotic thrombocytopenia purpura and renal failure (Cray and Moon, 1995). Thrombotic thrombocytopenia purpura and renal failure conditions associated with *E. coli* O157:H7 infections, most commonly occurs in children and immune compromised persons.

The kidneys are the end organs most severely affected, but ischemic necrosis of the intestines, central nervous system (stroke), and to any other organ may occur (Nataro and Kaper, 1998; Paton and Paton, 1998). Typically, the illness begins with abdominal cramps and then diarrhoea, which is initially watery but becomes grossly bloody within 2 – 3 days (Mead *et al.*, 1999; CFSPH, 2004). On approximation, 15% of persons with HUS die or develop chronic renal failures (Donnenberg and Whittam, 2001). The illness is usually self-limiting and lasts for an average of 8 days (CFSPH, 2004).

### 2.10.2 Gastrointestinal illness caused by *Escherichia coli* O157:H7

**As a pathogen, *E. coli* O157:H7 is known for its ability to cause intestinal diseases (Todar, 2002). *Escherichia coli* O157:H7 infection presents as mild non-bloody or severe bloody diarrhoea (hemorrhagic colitis) (Donnenberg and Whittam, 2001).**

**Patients infected with *E. coli* O157:H7 also experience severe abdominal pain, with little or no fever (Donnenberg and Whittam, 2001; CFSPH, 2004). Because of *E. coli* O157:H7 infections, peadiatric patients are particularly at risk of developing strokes, pancreatitis, colonic perforations, hypertension and coma may be noticed (CFSPH, 2004). Toxins produced by *E. coli* O157:H7 mediate symptoms that have been shown to have cytotoxic effects on Vero cells (Donnenberg and Whittam, 2001).**

## 2.11 HOSPITALIZATIONS AND DEATHS DUE TO *E. COLI* O157:H7

### *2.11.1 Hospitalizations and deaths due to E. coli O157:H7 in other parts of the world*

From 1982 to 2002, 350 outbreaks were reported from 49 states, accounting for 8,598 cases of *E. coli* O157 infection in the United States alone (Rangel *et al.,* 2005). Among these cases, there were 1,493 (17.4%) hospitalizations, 354 (4.1%) cases of HUS, and 40 (0.5%) deaths. An estimated 73,480 illnesses due to *E. coli* O157 infection cases are reported to occur each year in the United States, leading to an estimated 2,168 hospitalizations and 61 deaths annually (Mead *et al*., 1999; Rangel *et al.,* 2005). A total of 38 cases of *E. coli* O157:H7 were reported in Oklahoma in 2005, resulting in an incidence rate of 1.10 cases per 100,000 populations. Of the reported cases in Oklahoma, 12 (32%) were hospitalized, however, no deaths were reported. An outbreak of *E. coli* O157 infections was reported in the east of Scotland in 2006 whereby 13 cases were confirmed (NHS, 2006)

### *2.11.2 Hospitalizations and deaths due to E. coli O157:H7 in sub-Saharan Africa*

**Surveillance of *E. coli* O157:H7 infection is well established in many developed countries and it may be apparent that there are geographical differences in the incidences of infection. However, many African countries do not conduct routinely test for *E. coli* O157:H7, hence many infections may go unrecognized. This has created substantial gaps in knowledge about epidemiology of *E. coli* O157:H7 in developing countries.**

**Although reported outbreaks of *E. coli* O157: H7 in sub-Saharan Africa have been few, available information indicates the presence the pathogen within communities. *Escherichia coli* O157: H7 cases have been isolated in South Africa (Le Roux, 2001); Swaziland (Isaacson *et al.,* 1993), Malawi and Mozambique (Paquet *et al*., 1993); Central African Republic (Germani *et al.,* 1997) and Kenya (Arimi, 2000); Nigeria (Olorunshola *et al.,* 2000) and Ivory Coast (Dadie *et al.,* 2000). In Zemio, a small village located on the Democratic Republic of Congo border witnessed outbreaks of bloody diarrhoea in 1996, which was attributed to *E. coli* O157. In December 1997, an epidemic of bloody diarrhoea due to *E. coli* O157:H7 was reported in Ngoïla. The outbreak resulted in the deaths of 11 patients (Germani *et al*., 1998).**

## 2.12 *SALMONELLA* SPECIES

***Salmonella* is a bacterial genus, divided into two species, *Salmonella* bongori and *Salmonella* enterica. *S.* enterica itself is comprised of six subspecies: they are *S.* enterica subsp. enterica, *S.*enterica subsp. salamae, *S.* enterica subsp. arizonae, *S.* enterica subsp. diarizonae, *S.* enterica subsp. indica, and *S.*Enteric subsp. houtenae, or I, II, IIIa, IIIb, IV and VI, respectively (Porwollik *et al.,* 2003). Of these six subspecies, only subspecies I is**

*2.12.1 Salmonella in developing countries*

Literature data related to water-borne *salmonellae* in developing countries relate mostly the typhoid *Salmonella* serovars. In the less industrialized area of the world, particularly in the Indian sub-continent and South East Asia, typhoid and paratyphoid fevers occur both in epidemic and endemic form, and remain a major public health problem (DeRoeck *et al.*, 2007). The burden of typhoid fever worldwide is further compounded by the spread of multiple drug resistant *S*. Typhi (Kim, 2010). Most of the recent publications on typhoid and paratyphoid fever water-borne infections in developing countries are from the Asian continent (Bhunia *et al.,* 2009) was associated with living in a house without water supply from the municipal network along with the use of low quality water for washing and drinking (Gasem *et al.*, 2001). Finally, Sharma *et al.(*2009) showed that the unsafe use of water, namely scooping out water from containers instead of using piped water or water stored in a narrow mounted container at home, was associated with typhoid fever. Using GIS to explore the epidemiological pattern across Vietnam by investigating the risk factors associated with shigellosis, typhoid, and cholera, Kelly-Hope *et al.* (2007) revealed that typhoid fever prevailed in the Mekong River Delta. They

observed that typhoid fever was mostly associated with high vapor pressure and the use of river and streams as drinking water. On the contrary, forested regions and public tap drinking water were found to be negatively associated with typhoid fever. In contrast to this, Vollaard *et al.* (2004) showed that fecally contaminated drinking water was not associated with typhoid and paratyphoid fevers in Jakarta Indonesia. Vollaard *et al.* (2004) also showed that typhoid fever was significantly associated with consuming ice cubes and iced drinks highlighting this as a likely water mediated route of transmission. The importance of ice cubes as a potential source of typhoid infection is supported by other studies in which it was shown that consumption of ice cubes (Gasem *et al.*,2001),flavored ice drinks and ice creams (Luby *et al.*, 1998) are risk factors for this disease. Nevertheless most of the recent risk factor studies showed that use of unsafe water was not the only risk factor associated with typhoid/paratyphoid fever thus indicating multiple routes of transmission for these diseases (Gasem *et al.*, 2001; Sharma *et al.*, 2009). Interestingly, Vollaard *et al*. (2004) showed that in Jakarta typhoid and paratyphoid fevers are associated with different routes of transmission. Risk factors for typhoid and paratyphoid fevers respectively, were mainly within and outside the household.

As in other developing countries, in Africa, *Salmonella* Typhi is a major cause of enteric disease, particularly in children (Crump *et al.*, 2005). The real burden of typhoid fever in Africa is largely unknown because of the lack of comprehensive surveillance studies and credible measures of the disease incidence. However, a mean incidence of 50 cases per 100,000 persons per year has been estimated for the whole continent with a highest value in South Africa (N100/100,000 cases/year). (Crump *et al*., 2004).

In Nigeria, Oguntoke, *et al.,* (2009) investigated relationships between water-borne diseases, morbidity patterns, drinking water source quality and water handling practices in Ibadan City. Oguntoke *et al.,* found that typhoid fever was the most common water-borne disease (39.3%) in the area followed by bacillary dysentery and cholera. In agreement with the hypothesized water mediated route of transmission about 50% of the analyzed water-borne disease infections occurred in the heavy rain period (Oguntoke *et al.,* 2009). In Ibadan City, *Salmonella* Typhi and Paratyphi were detected in the well waters and the microbial water quality was found to be positively correlated with the pattern of water-borne disease within the selected areas. Additionally multivariate regression analysis showed that the percentage of residents applying inadequate domestic water treatment (mostly potash alum addition) explained 68.6% (Pb0.04) of the pattern of waterborne diseases morbidity in the area (Oguntoke *et al.,* 2009).

### *2.12.2 Physiological characteristics of Salmonella species*

***Salmonella*** **species are Gram negative rods. On blood agar, colonies are 2-3 mm in diameter. Colonies are generally non-lactose fermenters.** ***Salmonella*** **species are motile (with a few exceptions), facultatively anaerobic, produce acid from glucose usually with the production of gas, and are oxidase negative (Cheesbrough, 2006). Most produce hydrogen sulphide except** ***Salmonella*** **Paratyphi A and** ***Salmonella*** **Typhi, which is a weak producer. They are identified with a combination of serological and biochemical tests.**

***Salmonella*** **species are classified and identified into serotypes according to the Kauffmann White scheme which currently contains in excess of 2500 serotypes. Primary subdivision is into "O"sero-groups (those which share a common somatic antigen), and these are then subdivided on the basis of "H"(flagella) antigens(NCISM 1990). Strains of** ***Salmonella*** **Typhi may produce Vi antigen, which is an acidic polysaccharide layer outside the cell wall. When fully developed it renders the bacteria agglutinable with Vi antiserum and inagglutinable by "O"antiserum. Antigens similar to Vi may also be found in some strains of** ***Salmonella*** **Para-typhi C and** ***Salmonella*** **Dublin.**

### *2.12.3 Pathogenesis of Salmonella spp.*

***Salmonella*** **is one of the most important water transmitted bacteria because of the severe diarrhea and typhoid fever they caused with human and animal excreter acting as sources of the pathogen. These species cause a variety of infections both in humans and domestic animals, ranging from mild food poisoning such as gastroenteritis caused by** ***Salmonella*** **typhimurium to severe systematic disease called typhoid caused by** ***Salmonella*** **typhi (Baudert** ***et.,al*** **2000).*Salmonella* belongs to the family Enterobacteriaceae. They caused a wide range of human diseases such as enteric fever, gastroenteritis and bacteremia (Anonymous, 1995).**

#### *2.12.3.1 Salmonellosis*

**Salmonellosis is a disease caused by members of the genus *Salmonella* (Nietfeld and Kennedy, 1999). It is an important cause of diarrheal illness in humans, causing approximately 1.4 million illness and 600 deaths annually in the United States (Brenner *et al.,* 2000). Much of what is known about the epidemiology of salmonellosis comes from outbreak investigations. These investigations have determined that most human infections result from the ingestion of foods of animal origin that are contaminated with *Salmonella* species(Mead *et al.,*1999) being *Salmonella* of animal origin causes an intestinal infection, characterized by sudden onset of fever, myalgia, cephalagia, abdominal cramps, nausea and vomiting. There is increased risk of exposure among those who work in abattoirs, poultry processing plants and those in contact with animal and their products.**

#### 2.12.3.2 *Enteric fever*

**Typhoid fever is an important health problem in many developing countries. It causes over 20 million cases annually with at least 700,000 deaths (Cooke and Wain, 2004). The infection is caused by *S.*typhi and it occurs most frequently in children and young adults (3-19) years. Symptoms of enteric fever include persistent high fever with low pulse rate, severe headache, toxaemia, enlargement of the spleen, nausea, and apathy or mental confusion. The organisms multiply in reticuloendothelial cells. Invasion of the intestine causes inflammation and perforation, epistaxis, intestinal haemorrhage and perforation, toxaemia and renal failure may occur in untreated late typhoid. A rash on the trunk may be seen on light coloured skin. In uncomplicated typhoid, the total white cell count is normal or low with a relative lymphocytosis. There may be also anaemia. A sudden increase in white cell count may occur with intestinal perforation. Infection with S.typhi can also cause osteomyelitis and typhoid arthritis particularly in those with sickle cell disease and thalassaemia.(Cheesbrough, 2006).**

#### *2.12.3.2 Enterocolitis*

**This can be caused by many *Salmonella* serovars. In developing countries, *S.*Typhimurium and *S.*Enteritidis are common causes. Infection is by ingesting *Salmonellae* in food that has become contaminated from animal or human intestinal sources, directly or indirectly. Common sources of infection are poultry, meat and meat products, eggs and egg product. Diarrhoea, vomiting, fever and abdorminal pain occur 12-36hours after eating contaminated food.In acute infections, blood and mucous are present in faecal specimens. Infants and the elderly, persons in poor health, those with ulcerative colitis, malignancy and immunosuppressed persons are at greater risk of developing serious disease. (Cheesbrough, 2006).**

### *2.12.3.3 Bacteraemia*

**Non-typhi *Salmonellae (*NTS) particularly *S.*Typhimurium and *S.*Enteritidis are common causes of bacteraemia and septicemia in young children in developing countries. In Africa and elsewhere, NTS bacteraemia is also common in those coinfected with HIV. Others at high risk are the elderly in poor health, those with malignancy, sickle cell disease and chronic schistosomiasis.(Cheesbrough, 2006).**

### 2.12.4 Routes of transmission

**Contaminated food is the major source of transmission of salmonellosis (Guithrie, 1991). Frequency of occurrence is greatest in foods which are not cooked or have been incompletely cooked, such as roasted meat (in which suya and balangu belongs) or roasted chicken. Eating half cooked suya or balangu processed at road side under unhygienic condition is a common practice in Nigeria, there by exposing the consumers to *Salmonella* infections. *Salmonella* have also been documented in foods, water (Cheung *et al.,* 2004), poultry and poultry feeds (Eyigor and Carli, 2003), and shellfish (Kumar *et.,al* 2008). Others include eggs and egg products, poultry meat and meat from other food animals, and meat products. Contaminated salad crops, sprouted seeds, milk products and spices have also been involved in numerous outbreaks. *Salmonella* Enteritidis and *S.*Typhimurium are the most widespread serovars in many European countries (although *Salmonella* is rare in livestock in some EU countries because of their very strict control programmes). *Salmonella* Typhimurium is the dominant serovar in North America (Saeed *et al.,* 1999; Wray & Wray, 2000) it was also recognised in various animal species and humans in many countries in recent years (Soyer *et al.,* 2009).**

## 2.13 ANTIBIOTIC RESISTANCE

**Antibiotic resistance is forms of drug resistance whereby some sub populations of micro organisms usually a bacterial species are able to survive after exposure to one or more antibiotics; pathogens resistant to multiple antibiotics are considered multi-drug resistant (MDR) (CDC).**

**Resistance to antibiotics has resulted in morbidity and mortality from treatment failures and increased health care costs. Although defining the precise public health risk and estimating the increase in costs is not a simple undertaking, there is little doubt that emergent antibiotic resistance is a serious global problem.(Karlowsky *et al*., 2003).**

**Antimicrobial resistance in Enterobacteriaceae poses a critical public health threat, especially in the developing countries (WHO, 2008). Much of the problem has been shown to be due to the presence of transferable plasmids encoding multidrug resistance and their dissemination among different enterobacterial species (Blake *et al.,* 2003). *Escherichia coli* O157 are an important food-borne and water-borne pathogen with a worldwide distribution (Matthews *et al.,* 2013). The first reported outbreak of *E. coli* O157 infection in the developing world occurred in 1992 in Southern Africa (Effler *et al.,* 2001). Outbreaks have also occurred in Central African Republic in 1996 and Cameroon, in 1997 (Cunin *et al.,* 1999). Such outbreaks have been linked to contaminated bovine food products, contaminated drinking water and flood irrigation with water contaminated by animal faeces or surface run off and cattle faeces have been implicated as the major source of contamination (Effler *et al.,* 2001). The emergence of antimicrobial resistance is usually preceded by antimicrobial misuse; however, surveillance of the spread of antimicrobial-resistant pathogens is expected to play a very important role in reducing the rate of emergence and spread of antimicrobial-resistant pathogens since such early warning signals make timely intervention possible. Studies during the last 15 years shows increasing resistance to commonly used antimicrobials such as trimethoprim-sulphamethoxazole (TMP-SMX, also known as cotrimoxazole), ampicillin, tetracycline and chloramphenicol (Iwalokum *et al.,*2001). The use of antibiotics in medicine and their applications in animal husbandry has**

**brought about phenotypic changes, often due to chromosomal mutations, and antibiotic resistance in *E. coli* has been globally identified in isolates from environmental, animal and human sources (WHO, 2014). Kinge *et al*(2010). reported that *E. coli* has been linked to well-known antibiotic-resistant gene pools and that these genes are transferred into the normal flora of humans and animals, where they exert a strong selective pressure for the emergence and spread of resistance in *E. coli* strains. Inevitably, they discover their route into nature's turf through wastewater, compost and sewage slime. In much of the developing world without access to good quality medicines, infections continue to be the major killers, and in all countries, infections with resistant microorganisms are a major cause of death (WHO, 1999). Antimicrobial resistance is generally not a problem of disease pathology but one of limited therapy options (Samie *et al.,* 2012), thus containment strategies must be adapted to the needs of specific disease control and treatment programs. Antibiotic-resistant bacteria have been found in a surprisingly diverse range of environments, including clinics, animal pens, orchards, aquaculture, food, sewage as well as chlorinated and unchlorinated water supplies (Chopra and Roberts, 2001). Bacteria are common contaminants worldwide; and the release of human and animal wastes into the environment exacerbates bacterial contamination, especially of aquatic milieu. Increased resistance to antibiotics may pose a challenge for the effective treatment of bacterial infections (Fasehun, 1999). The collapse of primary healthcare system coupled with the unavailability of drugs in hospitals in this country has**

**resulted in most people resulting to purchasing drugs in some cases from road side sellers instead of going to the hospitals, thereby exposing themselves to the danger of acquiring multidrug resistant (MDR) microorganisms.**

**Like other developing countries, treatment of patients in Nigeria has been based on the use of first line (conventional) antibiotics, such as ampicillin, chloramphenicol and co-trimoxazole and the third generation cephalosporins. However, efficacies of some of these drugs have been doubtful, following the emergence of multidrug resistance in *Salmonella* strains (Akinyemi *et al.,* 2000). Fluroquinolones have been found to be efficacious both in-vitro and vivo in the treatment of several *Salmonella*-associated illnesses, although strains with reduced susceptibility to ciprofloxacin among travellers have been reported in some parts of the globe (Hakanen *et al.,* 2001).**

**Recently a monophasic variant of *S.* Typhimurium (S.4,5,12:i:-DT193 with resistance to ampicillin, streptomycin, sulphonamides and tetracycline) has emerged in pigs and caused outbreaks of salmonellosis in humans in several countries worldwide, and there are several other emergent monophasic *S.*4,5,12:i:- and *S.*4,12:i:- strains with varying resistance patterns that have been recognised in various animal species and humans in many countries in recent years (Soyer *et al.,* 2009).**

**Clinicians used antibiograms to assess local susceptibility rates, as an aid in selecting empiric antibiotic therapy, and in monitoring resistance trends over time within an institution. An antibiogram shows the aggregate number of bacteria tested against antimicrobials and incorporates the extent of bacterial isolates vulnerable to every antimicrobial operator tested (Fridkin *et al.,* 2001). They lend information that can be used to raise awareness about resistance problems, support the use of optimal empiric treatment, and identify opportunities to reduce inappropriate antibiotic usage and to discover success of such efforts (Van Beneden *et al.,* 2003).**

**The most common methods utilized to measure the *in vitro* vulnerability of microorganisms to antimicrobial operators include the disk diffusion method, agar dilution, broth micro-dilution, and testing by antimicrobial gradient agar strips (E-test method). The disk diffusion (qualitative test) method can be used to estimate the *in vivo* adequacy of numerous antibacterial agents by measuring the zone of inhibition diameters and comparing it to the standards described by CLSI. In clinical microbiology laboratories, the antimicrobial gradient method is used routinely for testing common, rapidly growing, and certain fastidious bacterial pathogens (NCCLS, 2003). The antimicrobial gradient diffusion method uses the principle of establishment of an antimicrobial concentration gradient in an agar medium as a means of determining susceptibility.**

***Escherichia coli* O157:H7 does not appear to have any increased resistance to antimicrobial food additives (Buchanan and Michael, 1997). Previous studies established that this bacterium has higher susceptibility to most antibiotics (Johnson *et al.*, 2000). Cattle treated with neomycin sulfate showed reduced shedding of *E. coli* O157:H7 (Callaway *et al.,* 2003). However, antibiotics are not recommended for control of *E. coli* O157:H7 due to fear of release of toxins into the systems of patients (Wong *et al.,* 2000). It is also thought that treatment with some antibiotics may lead to kidney complications (Walterspiel *et al.,* 2003). Nonetheless, these bacteria have high sensitivity to disinfectants. For instance, the use of 2% sodium hypochlorite, 70% ethanol, phenolic or iodine-based disinfectants, glutaraldehyde and formaldehyde has demonstrated lethal effect on these bacteria (CFSPH, 2004).**

# 3.0 CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 STUDY AREA

The study was carried out in Katsina State, Nigeria. The State has a total population of 5,801,584, going by the 2006 census (FGN 2007). Katsina State is bounded by Niger Republic to the north, by Jigawa and Kano States to the east, by Kaduna State to the South and by Zamfara State to the West. It has thirty four (34) Local Government Areas with 3 senatorial zones namely: Katsina South, Katsina Central and Katsina North**.** Katsina State is situated at 12.99$^{o}$ North latitude, 7.6$^{o}$ East longitude and 464m elevation above the sea level with two distinct seasons, wet and dry seasons. Three (3) Local Government Areas were selected from the State for this research, namely; Mani, Danmusa and Danja.




**Map of Katsina State Showing the Selected Areas for the Study.**
**Source:**- NASA/NOA Spot Image 2014.

**Figure 3.1**: **Administrative Map of the study area**

## 3.2 STUDY DESIGN

This study was a cross-sectional study.

## 3.3 SAMPLING METHOD

### *3.3.1 Sample Size*

This was calculated using the isolation rate of 2.2% as given by Chigor *et al.* (2010)

$$n = \frac{Z^2pq}{l^2}$$

Where,

Z= 1.96 for 95% confidence interval

P= Prevalence rate

q=1-P

l= allowable error (5%)

n=number of samples

$$= \frac{(1.96)^2 \times 0.022 \times 0.978}{(0.05)^2}$$

= 33.06236

Approximately 33 samples but inorder to obtain a representative sample, one hundred (100) samples were collected from each of the three local governments in the three senatorial zones, making a total of 300 samples.

## 3.4 SAMPLING PLAN

*3.4.1 Type of sample: well water*

*3.4.2 Sample size: 300*

*3.4.3 Sample Collection*

Three hundred (300) well water samples were collected using simple random sampling technique in sterile 250ml containers, the collected samples were labeled and transported to the laboratory of the Department of Microbiology Ahmadu Bello University, Zaria, Nigeria in ice packs and were analysed within 6 hours (APHA, 2011).

Wells were observed for the presence or absence of well casing, well covers and distance from the latrine or soak away using a tape rule (Egwari and Aboaba, 2002).

## 3.5 SAMPLE ANALYSES

Parameters such as temperature, pH, appearance (turbidity) and odor were observed at point of collection, using thermometer and a standard pH meter respectively, turbidity was determined using the Naphelometric method, and odour was determined using the sense organ for smelling.

3.5.0 *Bacteriological analysis of the water samples*

*3.5.1 Enumeration of coliforms*

A series of lactose broth tubes ware inoculated with measured amounts of the water samples to be tested (10ml,1ml and 0.1ml respectively). The tubes were incubated at $35^0$C for 24-48 hours. Gas production, acid reaction and abundant growth in any one of the tubes is presumptive evidence of the presence of coliforms. Tubes with positive presumptive reaction were subsequently subjected to confirmatory test using an E.coli

medium. The most probable number (MPN) of coliforms in 100 ml of the water samples were estimated by the number of positive tubes.

*3.5.2 Total plate count*

Ten-fold serial dilutions of water samples were prepared using sterile distilled water as a diluent. From each dilution, 0.1ml was spread aseptically onto duplicate plates of Eosin methylene blue agar (Oxoid) following standard procedure, the plates were incubated at $37^0$ C for 24 hours. Typical lactose fermenting colonies 0.1-0.5mm pinkish coloured were counted and reported as cfu/ml and the value multiplied by the dilution factor to get the correct cfu/ml.

*3.5.3 Isolation of Escherichia coli using streak plating method*

One ml of the water samples was pre-enriched in nine ml of double strength tryptone soy broth and incubated at 37ºC for 24 hours. Then 0.1 ml of the broth that shows an evidence of growth after incubation was sub-cultured on EMB agar and further incubated at 37ºC for 24 hours. Green metallic sheen colonies with dark centers were gram stained and stored on nutrient agar slants for further characterization by employing biochemical tests.

3.5.4 *Isolation of Salmonella spp using streak plating method*

One (1ml) of the sample was pre-enriched into 10mls Selenite F broth and incubated overnight at 37ºC, then 0.1ml of the broth was streaked on *Salmonella-Shigella* agar and

further incubated at 37ºC for 24 hours. Suspected *Salmonella* colonies were Gram stained, purified and stored on nutrient agar slants for further characterization.

### *3.5.5 Gram Staining and Microscopy*

Gram staining reaction is used to help identify pathogens in specimens and cultures by their Gram reaction (Gram positive or Gram negative) and morphology. The required Gram staining reagents that were used included; Crystal violet stain, Lugol's iodine, Acetone-alcohol decolourizer, and safranin. Dried smear of the isolate obtained was heat-fixed and stained with crystal violet solution (primary dye) for 60 seconds, rinsed with tap water and drained to avoid diluting with the mordant. It was further flooded with iodine solution (mordant) for 30seconds and rinsed. Decolourizer was applied drop wise on the tilted slide until all the colour of the dye was removed and subsequently rinsed with tap water. The slides were further flooded with safranin for 60seconds, rinsed and air dried. Finally, all slides were examined under the microscope at x100 objective lens after applying a drop of immersion oil (Cheesbrough, 2006).

### 3.5.6 Biochemical Tests

#### *3.5.6.1 Indole test*

The isolates were inoculated into 5mls of indole medium and incubated at 37°C for 24 hours. After incubation, few drops of Kovac's reagent were added and shaken gently. The absence of red colour in the reagent layer above the broth indicated a negative test while the dissolution of indole in the reagent which became red and form a layer above the surface of the broth indicated a positive test (Cheesbrough, 2004).

*3.5.6.2 Methyl red test*

The isolates were inoculated onto 5mls of MRVP broth then were incubated at 37°C for 24 hours. After incubation, 1ml of the broth was transferred to a small tube and 2-3 drops of methyl red indicator were added. Appearance of a red colour indicated a positive test (Singleton, 1997).

*3.5.6.3 Voges Proskauer test*

To the rest of the inoculated and incubated MRVP broth used above, 1-2 drops of α-napthol reagent was added and followed by 1-2 drops of 40% KOH. The broth was shaken and the cap of the tube was loosed and placed in a slanting position. The absence of a red colour from the liquid interface within 30-60 minutes indicated a positive test (Singleton, 1997).

*3.5.6.4 Citrate utilisation test*

The isolates were inoculated into Simmon's citrate agar slants and incubated at 37°C for 24 hours. The development of a bright blue colour indicated a positive result (Cheesebrough, 2004).

*3.5.6.5 Oxidase test*

A piece of filter paper was moistened with few drops of oxidase reagent, a colony of the test organism was smeared on the filter paper. A deep purple colour indicated a positive result (Cheesebrough, 2004).

*3.5.6.6 Urease test*

The test organism was cultured in a medium containing urea and phenol red indicator, it was incubated at 37°C for 24 hours. Pink colour indicated a positive test (Cheesebrough, 2004).

*3.5.6.7 Motility test*

Motility medium was prepared and dispensed in tubes, a straight wire or inoculating needle was used to obtain an innoculum and was stabbed into the medium, the tubes were incubated at 37°C for 24 hours. A visible growth extension away from the inoculation line indicated a positive test (Cheesebrough, 2004).

*3.5.6.8 Lysine Decarboxylase Test*

A dense suspension of the test organism was prepared in 0.25ml physiological saline in small tubes, an L.D.C tablet was added with 3 drops of paraffin oil, the tubes were incubated at 37°C for 24 hours. A deep blue or violet colour indicated a positive test, while yellow, green or gray indicated a negative test (Cheesebrough, 2004).

*3.5.6.9 Carbohydrate fermentation test in Kligler Iron Agar.*

Kligler Iron Agar (KIA) medium was prepared and dispensed in tubes, an inoculating needle was used to obtain an inoculum and stabbed into the medium, the tubes were incubated at 37°C for 24 hours. *Salmonella* spp produced a pink-red slope and yellow butt with blackening due to production of hydrogen sulphide and cracks due to gas production from glucose fermentation. While a positive test for *E.coli* was indicated by acid reaction with gas production (Cheesebrough, 2004).

*3.5.6.10 Sugar (Carbohydrate) fermentation test*

The carbohydrate fermentation test was performed by inoculating a loopful of a test bacterial culture into the individual tubes containing 1%sugars which include; dextrose, maltose, sucrose, lactose, mannitol and dulcitol and were incubated at 37°C for 48 hours. Acid production was indicated by the change of media from pink to yellow colour while gas production was indicated by the appearance of gas bubbles in the inverted Durham's fermentation tubes (Cheesebrough, 2004).

*3.5.7 Isolation and confirmation of E. coli O157:H7*

Confirmed *E. coli* were streaked on Sorbitol MacConkey agar. Those that were colourless were tested with latex agglutination kit for *E.coli* O157: H7. The positive isolates were taken as *E.coli* O157:H7 while those that did not show agglutination were taken as non - *E.coli* O157:H7.

*3.5.7 Isolation and confirmation of Salmonella spp.*

Oxoid Rapid *Salmonella* Antibody agglutination was performed by mixing a loopful of suspected colonies in to a polyclonal antibody mixture for ten seconds on a card, which is then tilted back and forth for an additional minute as per manufacturer's instructions. Agglutination of the beads signifies a positive test for *Salmonella* spp.

*3.5.8 Serogrouping of positive Salmonella isolates*

The positive *Salmonella* isolates were further sero-grouped using *Salmonella* sero-quick grouping kit (Copenhagen, Denmark). It was done by placing a drop of sterile normal saline on a clean slide and mixed with a loop of the *Salmonella* isolate followed by adding a drop of the antiserum, the slide is then tilted back and forth for a minute

according to the manufacturer's instructions. Agglutination reaction indicated a positive result for each specific antiserum used.

### *3.5.9 Antibiotics susceptibility Testing*

Each of the isolate was subjected to antibacterial susceptibility testing using the Kirby-Bauer (1996) method that has been standardized and evaluated by the methods of Clinical and Laboratory Standards Institute 2012. *E. coli* ATCC 25922 was used as a control strain which was obtained from National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria.

The susceptibility testing was done by the disc diffusion technique on Mueller Hinton agar. The organisms were tested in vitro for susceptibility to the following commonly used antibacterial drugs:

ceftaxidime(30µg),cefuroxime(30µg),gentamicin(10µg),cefixime(5µg),ofloxacin(5g),ciprofloxacin(5µg),augmentin(30µg),tetracycline(30µg),cefotaxime(30µg),chloramphenicol(30µg),cefoxitin(30µg)&sulphametazoletrimethoprim(25µg).

The isolates which have been freshly grown on nutrient agar were picked and emulsified in sterile normal saline. The prepared turbidity was matched with a turbidity standard (0.5 McFarland). A sterile cotton swab dipped into the standardized innoculum was used to streak the entire surface of dried Mueller Hinton Agar plates. Antibacterial Sensitivity disks were placed aseptically using a sterile forcep, and all plates were incubated at 37$^{o}$C for 24hrs (Mills –Robertson *et al*., 2003).

The results were interpreted using CLSI (2012) Guide lines. Each isolate was classified as;

1. Susceptible if the observed zone of inhibition diameter is equal or greater than CLSI sensitive diameter (mm)
2. Resistant if the observed zone of inhibition diameter was less than or equal to the CLSI resistant diameter (mm)

### 3.6 MAR INDEX.

The multiple antibiotic resistance (MAR) index was determined by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics tested (Olayinka *et al*., 2004).

$$\text{MAR index} = \frac{\text{Number of Antibiotics Isolate is Resistant to}}{\text{Total Number of Antibiotics Tested}}$$

### 3.7 STATISTICAL ANALYSES.

The data obtained from this study were subjected to statistical analysis. $Chi^2$- test and Odds ratio was used to determine the level and the strength of association at 95% confidence interval and at 0.05 significance level (SPSS, version 19), and other results were summarized into tables and charts.

# CHAPTER FOUR

## 4.0 RESULTS

Tables 4.1and 4.2 show result for mean coliform and mean aerobic plate counts of the well water samples obtained from the three(3) study locations respectively. Dan-musa Local Government Area had the highest mean coliform counts of 20.7cfu/ml and mean aerobic plate counts of 3.47±0.02, followed by Mani with19.7cfu/ml and mean aerobic plate counts of 2.88±0.02 and Danja Local Government Area had the least mean aerobic and coliform counts of 1.99±0.02 and 13.8cfu/ml respectively. The result showed that none of the water samples met the WHO (2006; 2008 and 2011) guidelines for drinking water quality, which states that the coliform count in drinking water both piped and unpiped should be 0 cfu/ml and 10cfu/ml (NAFDAC, 2007). Dan-musa Local Government Area is the worst affected area being with the highest mean coliform and aerobic plate counts while Danja Local Government Area is the least affected area with low coliform and aerobic plate counts.

Table 4.3 shows results of the physicochemical analysis of the water sampled from the 300 dug-wells. The pH of the water samples ranged from 6.9 to 7.3. The measured pH indicated that all the samples had values that were within the WHO (2004) recommended range of 6.5-9.5 for drinking water, and did not appear to pose any problem to the water. The temperature of the well water samples had a range from18-22$^{o}$C (Table 4.3). Although there is no guideline value set for drinking water by the Nigerian Environmental Protection Agency, the WHO recommends a range of 22 to 29$^{o}$C. Higher water temperatures are not recommended mainly because they make drinking difficult.

Turbidity values range from 2.0 NTU to 14.0 NTU which exceeds the WHO limit of 5NTU (Table 4.3). High turbidity in some of these wells could be because of soil disturbance and re-suspension within the wells during water withdrawal.

Most of the samples collected were clear with unobjectionable odour except some few wells in Dan-musa Local Government Area with slight odor which was against the limit set by (WHO, 2004).

Table 4.4 shows the distribution of presumptive *E. coli* and *Salmonella* species from the three Local Government Areas. Mani Local Government Area had the highest occurrence of *E.coli* isolates with 89.0 % (89/100) and *Salmonella* sp. 22.0% (22/100) followed by Dan-musa with *E.coli* 85% (85/100) and *Salmonella* sp. 12.0% (12/100) and Danja with the least frequency of occurrence of presumptive *E.coli*, 72.0% (72/100) and 9.0% (9/100) *Salmonella* spp. Well 3 in Dan-musa Local Government Area is the only well in which both *E.coli* O157:H7 and *Salmonella* sp.were isolated. The differences in the distribution as observed showed that there is significant statistical association with ($\chi^2$=10.705, df=2, P=0.005) for *E.coli* and *Salmonella* (**$\chi^2$=0.189, df=2, P=0.910).**

**4.1 Mean coliform count of the well water samples obtained from the (3) study locations in Katsina State.**

| Location | No. of samples | Mean coliform count(cfu/ml) | SEM |
|---|---|---|---|
| Danmusa | 100 | 20.7 | 0.0012 |
| Mani | 100 | 19.7 | 0.0134 |
| Danja | 100 | 13.8 | 0.0428 |
| Total | 300 | 54.2 | 0.0574 |

**Key: cfu=Colony forming unit, SEM= Standard error mean**

**Table 4.2: Mean viable plate counts of well water samples obtained from the three Local Government Areas in Katsina State.**

| **Sample location** | **No. of Samples** | **Mean±SEM ($log_{10}$cfu/ml)** | p-value |
|---|---|---|---|
| Dan-musa | 100 | 3.47±0.02 | 0.01 |
| Mani | 100 | 2.88±0.02 | |
| Danja | 100 | 1.99±0.02 | |
| Total | 300 | 2.78±0.02 | |

F= 7.162, Df=2, P-value 0.01

**Key**: SEM=Standard error mean, df= Degree of freedom, cfu=Colony forming unit

**Table 4.3:Physicochemical parameters of well water samples from the study locations in Katsina State.**

| Location | Sample Code | Temp. (°C) | pH | Turbidity (NTU) | Odour |
|---|---|---|---|---|---|
| **Mani** | Mn6 | 19 | 7.3 | 5.0 | Odourless |
| | Mn22 | 21 | 7.0 | 6.2 | Odourless |
| | Mn67 | 20 | 7.2 | 3.5 | Odourless |
| | Mn69 | 21 | 7.0 | 2.5 | Odourless |
| | Mn79 | 20 | 7.1 | 2.0 | Odourless |
| | Mn84 | 22 | 7.3 | 6.0 | Odourless |
| **Dan-musa** | Dm3 | 20 | 7.1 | 14.0 | Odour |
| | Dm21 | 20 | 7.0 | 2.0 | Odourless |
| | Dm38 | 20 | 7.2 | 4.5 | Odour |
| | Dm47 | 19 | 7.1 | 5.0 | Odourless |
| | Dm53 | 21 | 6.9 | 3.0 | Odourless |
| | Dm75 | 22 | 7.2 | 6.5 | Odourless |
| | Dm82 | 21 | 7.1 | 2.5 | Odourless |
| **Danja** | Dj9 | 20 | 7.2 | 14.0 | Odourless |
| | Dj11 | 18 | 7 | 3.0 | Odourless |
| | Dj66 | 19 | 7.2 | 5.0 | Odourless |
| | Dj69 | 19 | 7.1 | 12.0 | Odourless |
| **WHO Standard (2004 and 2006)** | | **25** | **6.5 – 9.5** | **5.0** | **Odourless** |

**Keywords: Mn=Mani, Dj= Danja, Dm= Dan-musa, NTU= Naphelometric turbidity units, Temp= Temperature**

**Table 4.4: Distribution of Presumptive *E. coli* and *Salmonella* Species in the well water obtained from the Study Areas in Katsina State.**

| Location | Number(%) of *E. coli* | Number(%) of *Salmonella spp* |
|---|---|---|
| Danja | 72 (72.0) | 9 (9.0) |
| Dan-musa | 85 (85.0) | 12 (12.0) |
| Mani | 89 (89.0) | 22 (22.0) |
| **Total** | **246 (82.0)** | **43 (14.3)** |

**($^{E.coli}$ $\chi^2$=10.705, df=2, P=0.005; $^{Salmonella}$ $\chi^2$=0.189, df=2, P=0.910)**

Table 4.5a shows results for serological identification of *E. coli* O157:H7 latex aggulutination test. Out of the 246 *E. coli* isolates, 7 were serologically found to be O157:H7

Table 4.5b shows result for serological identification of *Salmonella* isolates. Out of the 43 isolates 11 were found to belong to the genus *Salmonella*.

Table 4.6 shows result for sero-grouping of *Salmonella* isolates, 5(45.4%) were found to belong to sero-group D.(*S*.Typhi), 2(18.2%) belongs to sero-group A and B(ParatyphiA and Typhimurium) respectively, and lastly 2(18.2%) isolates were non-typable.

Table 4.7 shows the percentage distribution of *E. coli* O157:H7 and *Salmonella* serovars in the three Local Government Areas screened. Dan-musa Local Government Area had the highest occurrence of *E. coli* O157:H7 (57.1%) and *Salmonella* (36.4%) followed by Mani with 28.6% *E. coli* O157:H7 and 36.4% respectively. Danja had the least frequency of 14.4% *E. coli* O157:H7 and *Salmonella* 27.2%.

**Table 4.5a: Serological Identification of *E.coli* O157:H7**

| No.(%) | OB | INF |
|---|---|---|
| 7(2.9) | Agglutination | Positive |
| 239(97.1) | No Agglutination | Negative |
| **Total 246(100)** | | |

**Key: OB- Observation, INF- Inference, No.(%)-Number and percentage of isolates analysed.**

**Table 4.5b: Serological identification of *Salmonella* isolates**

| No.(%) | OB | INF |
|---|---|---|
| 11(25.6) | Agglutination | Positive |
| 32(74.2) | No Agglutination | Negative |
| **Total 43(100)** | | |

**Key: OB- Observation, INF- Inference, No.(%)Number and percentage of isolates analysed**

**Table 4.6: Sero-Grouping of *Salmonella* Isolates.**

| ***Salmonella* sero-groups (n=11)** | | | |
|---|---|---|---|
| **Group D (*S.* Typhi %)** | **Group A (*S.* Para-typhi A%)** | **Group B (*S.* Typhimurium %)** | **N.T. (%)** |
| 5 (45.4) | 2 (18.2) | 2 (18.2) | 2 (18.2) |

**Key: N.T- non-typable**

**Table 4.7: Percentage Distribution of *E.coli* O157:H7 and *Salmonella* Serovars from the Three Local Government Areas in Katsina State.**

| **L.G.A** | ***E. coli* O157:H7** | ***Salmonella* serovars** |
|---|---|---|
| Dan-musa | 4 (57.1) | 4 (36.4) |
| Danja | 1 (14.3) | 3 (27.2) |
| Mani | 2 (28.6) | 4 (36.4) |
| **Total** | **7 (100)** | **11 (100)** |

Table 4.8 shows result of the measured distances of wells positive for *E. coli* O157:H7 and *Salmonella* spp. from pit latrines, the distances were between 3-16m, which were all below the limit set by WHO(2006) and that of Nigerian Environmental Protection Agency of 30m each and 15.24m of the United State Environmental Protection Agency(USEPA). Most of the wells were uncovered while some were partially covered with woods or old rusted roofing sheets.

Table 4.9a shows the association between distance of wells from pit laterines and occurrence of *E. coli* O157:H7. Significant association was found ($p<0.05$). The strength of the association (Odds ratio) was also determined (2.741). This showed that *E.coli* O157:H7 is two times more likely to be found in wells with close proximity to pit laterines.

Table 4.9b shows the association between occurrence of the *Salmonella* serovars isolated and distance of wells from pit laterines. Significant association was also found ($p<0.05$), the strength of the association was further determined (odds ratio). The odds ratio for the three(3) sero-groups were determined, but that of *S.* Typhi was the highest (2.908), this shows that *S.* Typhi is two times more likely to be found in wells with close proximity to pit laterines than the other two (2) sero-groups.

**Table 4.8a: Distance between pit laterines and wells positive for *E. coli* O157: H7 in Dan-musa, Danja and Mani Local Government Areas of Katsina State.**

| Location | Well Identity | Distance (m) |
|---|---|---|
| Dan-musa | Dm3 | 3 |
| | Dm21 | 6 |
| | Dm38 | 5 |
| | Dm47 | 3 |
| Mani | Mn79 | 3 |
| | Mn84 | 9 |
| Danja | Dj9 | 4 |

**Keywords: Mn=Mani, Dj = Danja, Dm = Dan-musa**

**Table 4.8b:Distance between pit laterines and wells positive for S*almonella* Species. In Dan-musa, Danja and Mani Local Government Areas of Katsina State.**

| Location | Well Identity | Distance (m) |
|---|---|---|
| Dan-musa | Dm3 | 3 |
| | Dm53 | 12 |
| | Dm75 | 16 |
| | Dm82 | 10 |
| Mani | Mn6 | 7 |
| | Mn22 | 6 |
| | Mn67 | 8 |
| | Mn69 | 6 |
| Danja | Dj11 | 4 |
| | Dj66 | 7 |
| | Dj67 | 13 |

**Keywords: Mn=Mani, Dj = Danja, Dm = Dan-musa**

**Table 4.9a: Association between occurence of *E.coli* O157:H7 and distance of wells from pit laterines in Dan-musa, Danja and Mani Local Government Areas of Katsina State.**

| DISTANCE (m) | *E. coli* O157:H7 |
|---|---|
| 1-5 | 5 (71.4) |
| 6-10 | 2 (28.6) |
| 11-15 | - |
| >15 | - |

**Table 4.9b: Association between the *Salmonella* serovars isolated and distance of wells from pit laterines in Dan-musa, Danja and Mani Local Government Areas of Katsina State.**

| Distance (m) | *Salmonella* Serovars | | | | Total |
|---|---|---|---|---|---|
| | ***S.* Typhi** | ***S.* Paratyphi A** | ***S.* Typhimurium** | **Non typable** | |
| 1 - 5 | 1 | 1 | 0 | 0 | 2 |
| 6 – 10 | 4 | 1 | 1 | 0 | 6 |
| 11 – 15 | 0 | 0 | 0 | 2 | 2 |
| >15 | 0 | 0 | 1 | 0 | 1 |
| **Total** | **5 (45.4%)** | **2 (18.2%)** | **2 (18.2%)** | **2 (18.2%)** | **11 (100)** |

**($\chi^2$=27.343, df= 12, p= 0.007; OR= 2.908, 95% CI= 1.130-7.481)**

Figure 4.1 shows the results for antibacterial susceptibility testing. A total of 246 *E.coli* isolates were isolated and 7 out of them were confirmed to be *E.coli* O157:H7 using *E.coli* latex agglutination kit (oxoid, UK). Also 11 *Salmonella* isolates were isolated belonging to three (3) different sero-groups (D, A and B).

Antibiotic susceptibility profiles showed that virtually all the isolates were resistant to multiple antibiotics at various percentages.(Figure 4.1) However, 7 (100%) of the *E.coli* O157:H7 isolates were resistant to augmentin and tetracycline, 86% resistant to cefuroxime, sulphametazole-trimethoprim and gentamicin, 72% resistant to cefixime. However, ceftazidime, chloramphenicol, and floxacin were 100% effective against the organisms, cefotaxime and ciprofloxacin were 72% and 71% effective to the tested isolates respectively.

The 11 *Salmonella* isolates were tested against eight (8) different antibacterial drugs namely; cefoxitin 30µg, ceftazidime 30µg, ciprofloxacin 5µg, chloramphenicol 30µg, ofloxacin 5µg, tetracycline 30µg, and cefotaxime 30µg (Figure 4.2).ceftaxidime, tetracyclin and cefotaxime had the highest resistance, while resistance to ciprofloxacin was only observed in *S*. Typhi, but all the 11 isolates were sensitive to chlorampenicol, cefoxitin and ofloxacin (Figure 4.2). *S*. Typhi was the serotype with the highest percentage resistance of 62.5% among the strains isolated; the isolates were resistant to five (5) different antimicrobial drugs, while *S.* Paratyphi A and *S*. Typhimurium had the least percentage antimicrobial resistance of 37.5%. All the serotypes isolated were found to be resistant to more than one (1) of the tested antimicrobials. The most common resistance was to ceftazidime, cefotaxime and tetracycline (100%) respectively, while resistance to ciprofloxacin was only observed in *S*.Typhi with (33.3%). However, resistance to fluoroquinolones (ofloxacin was not encountered while reduced susceptibility of the isolates to

ciprofloxacin was observed (66.7%). However, all the serotypes were 100% sensitive to third generation cephalosporins (cefixime).

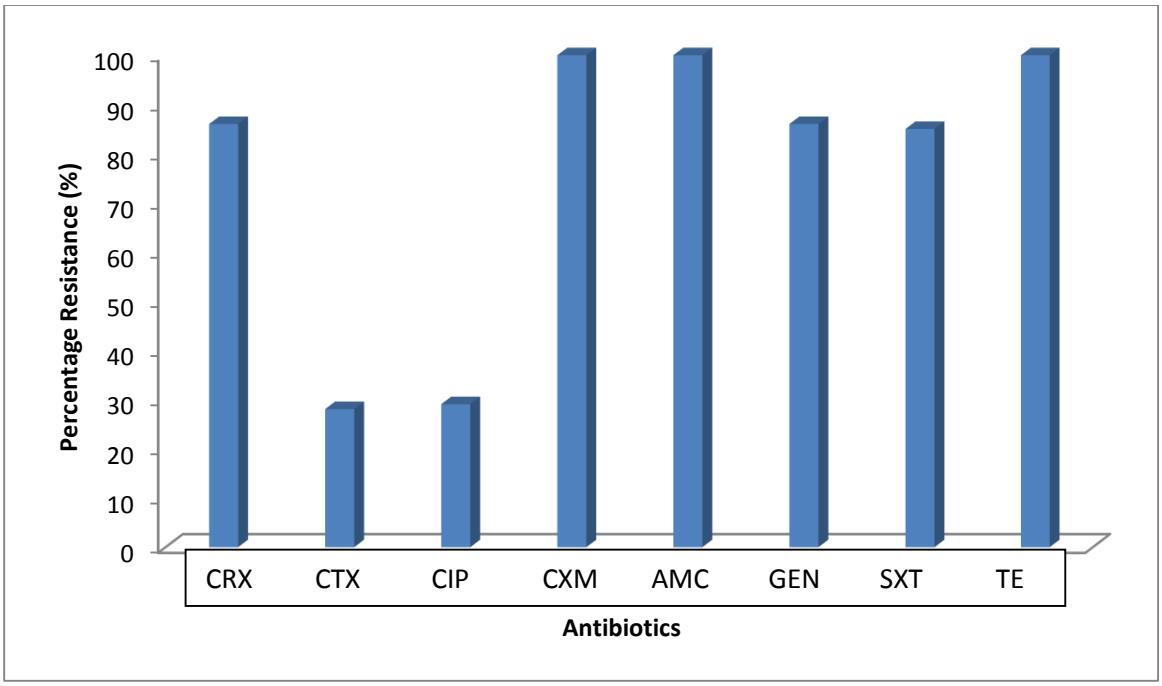



**Figure 4.1 Percentage antibacterial resistance of *E.coli* O157:H7**

**KEY: CRX= Cefuroxime, CTX= Cefotaxime, CIP= Ciprpfloxacin, CXM= Cefixime, AMC= Augmentin, GEN= Gentamicin, SXT= Sulphamethazole-trimethoprim, TE= Tetracyclin**

(Fig.4.2) Multiple antibiotic resistance (MAR) were determined for the *E. coli* O157:H7 isolates. Eight resistant phenotypes were obtained with varying combinations of 5,6,7 and 8. The predominant MAR phenotypes were CTX, AMC, GEN, TE, CXM having 41.0% and CRX, AMC, GEN, TE, CXM, SXT having 50.0% (Table 4.11).

Fig 4.3 shows the percentage antibiotic resistance for *Salmonella* isolates. Multiple antibiotic resistance (MAR) were also determined for the *Salmonella* serovars (Fig 4.4). Five resistant phenotypes were obtained with varying combinations of 3,4 and 5. The predominant MAR phenotypes were CAZ,CTX,TE having 38.0% and CAZ,CIP,TE,CTX having 50.0% and CAZ,F,CIP,TE,CTX 67% (Table 4.12).

**Table 4.11 Multidrug Resistance Pattern of *E.coli* O157:H7 showing the resistant isolates**

| S/N | No. of Resistant Isolates | Resistace pattern |
|---|---|---|
| 1 | 2 | CTX,AMC,GEN,TE,CXM |
| 2 | 3 | CRX, AMC,GEN,TE,CXM,SXT. |
| 3 | 1 | CRX,AMC,CIP,GEN,TE,CXM,SXT |
| 4 | 1 | CRX,CTX,AMC,CIP,GEN,TE,CXM,SXT |

KEY:AMC=Augmentin, GEN=Gentamicin, CIP=Ciprofloxacin, TE=Tetracycline, SXT= Sulfamethoxazole-trimethoprim, CXM=Cefixime, CRX=Cefuroxime, CTX=Cefotaxime.

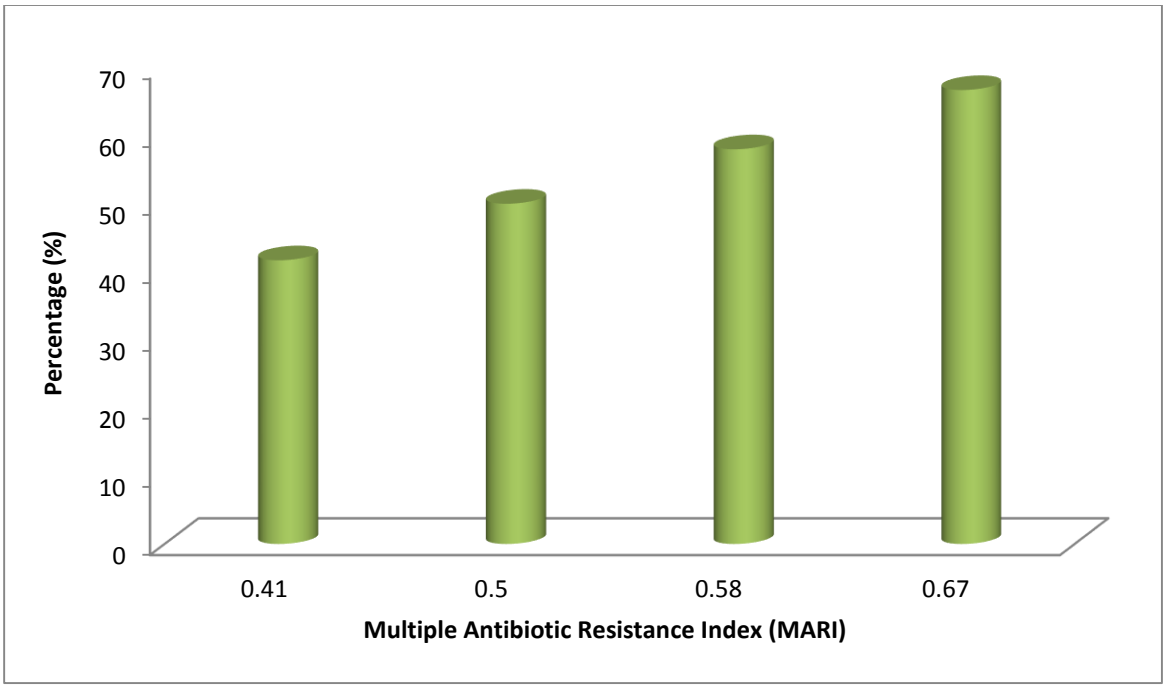



**Figure 4.2 Multiple Antibiotic Resistance Indices and the Percentage of *E. coli* O157:H7 Isolates from well water samples in Dan-musa, Mani and Danja Local Government Areas of Katsina State.**

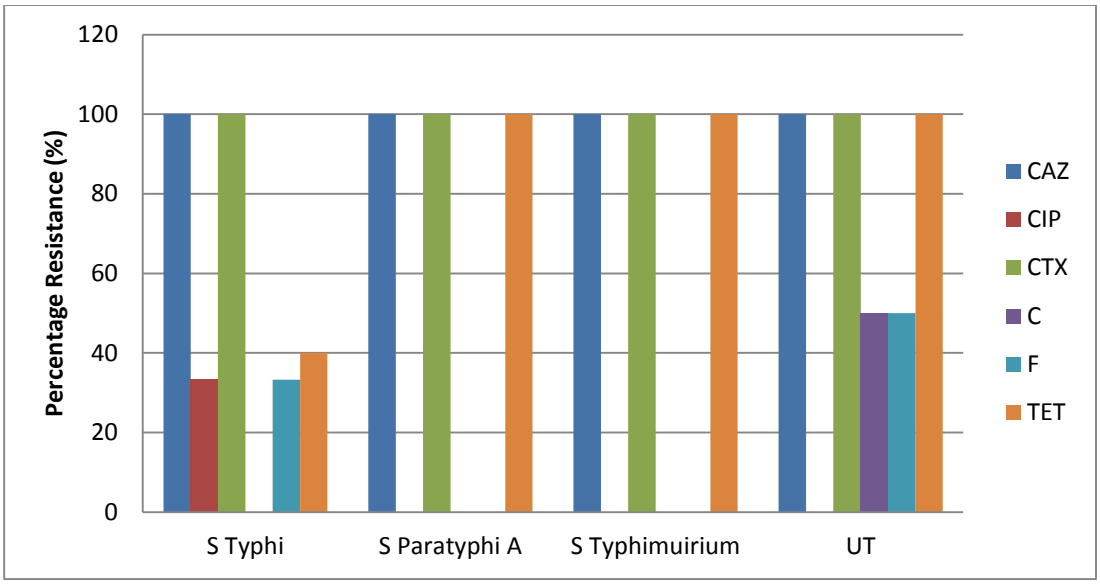



Fig 4.3 Percentage Antibiotic Resistance of *Salmonella* isolates

Key: CAZ= Ceftazidime, CIP= Ciprofloxacin, CTX= Cefotaxime, C= Chlorampenicol, F= Nitrofurantoin, TE= Tetracycline.

**Table 4.12 Multidrug Resistance Pattern of *Salmonella* serovars showing the resistant isolates**

| Organisms | No. of resistant Isolates | Resistant pattern |
|---|---|---|
| *S.* Typhi | 1 | CAZ, TE, CTX |
| | 2 | CAZ,CIP,TE,CTX |
| | 1 | CAZ,TE,CTX |
| | 1 | CAZ,F,CIP,TE,CTX |
| *S.* ParatyphiA | 2 | CAZ, TE,CTX |
| *S.* Typhimurium | 2 | CAZ, TE,CTX |

**KEY: CIP=Ciprofloxacin, TE=Tetracycline, CTX=Cefotaxime, CAZ=Ceftazidime,F=Nitrofurantoin.**

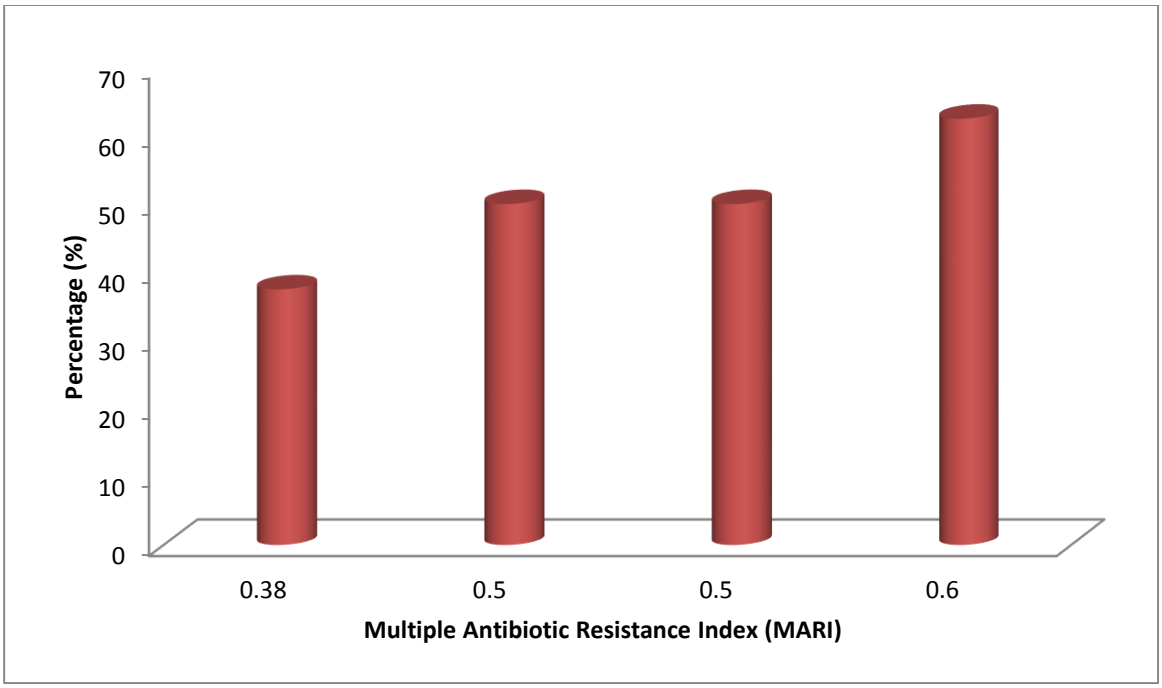



**Figure 4.4 Multiple Antibiotic Resistance Indices and the Percentage of *Salmonella* serovars isolated from well water samples in Dan-musa, Mani and Danja Local Government Areas of Katsina State.**

# CHAPTER FIVE

## 5.0 Discussion

The absence of municipal piped water has forced residents of the study area to rely on untreated ground water sources such as wells commonly dug by inhabitants. Results of this study showing that the water samples failed to meet the WHO (2006; 2007; 2011) standards for microbial contamination is not surprising since the commonest causes of ground water pollution can be easily observed in these locations. These activities include exposure to contamination due to human activities, the wells are often not covered and residents could be found washing clothes and dirty kitchen wares and sometimes bathing around the wells. Also animals/livestock are reared within compounds and households of owners, and are allowed to roam freely in search for food and water, hence they consequently serve as sources of faecal contamination of water sources (Olson, 2004).

Coliform bacteria have been widely used as indicator of the microbiological quality of surface and ground waters (Ahmed *et al.*, 2005). Thus the presence of coliforms is an index of bacteriological quality of water. The isolation of coliforms especially *Escherichia coli*, from the water sources is attributable to contamination by material of human and animal origin and this is of health significance as this organism have generally been reported as causative agent of gastroenteritis in humans. From the coliform count results in this study, none of the water simples met the WHO standard for drinking water which states that the coliform count in drinking water both piped and unpiped should be 0 cfu/ml and 10cfu/ml (NAFDAC, 2007). All the water sampled had very high counts and this indicates that they are unfit for human consumption.

Results of the physicochemical analyses obtained showed that temperature and pH of the wells analysed were all within the limit set by WHO (2004; 2006; 2011), but the turbidity values obtained have exceeded the WHO standard of 5NTU. High turbidity can stimulate bacterial growth, which indicates problems with treatment processes (WHO, 2004). It was also observed that the rope of the receptacle for water withdrawal is usually left in the dirty water around the dug-wells after water withdrawal and re-introduced into the wells during the next withdrawal. Turbidity is also considered as surrogate microbiological parameter because it is closely linked to the microbiological safety of drinking water. Turbidity can indicate that water may be contaminated with pathogens presenting human health concerns (Olson, 2004).

The presence of these pathogenic bacteria (*E.coli* O157:H7 and *Salmonella* spp.) in the untreated well water samples as obtained in this study is a clear indication of faecal contamination of the water source.

It was generally observed that wells located close to pit latrines are the wells positive for the presence of these pathogenic bacteria (*E. coli* O157:H7 and *Salmonella* species). This finding corroborates that of Sudgen (2006) who stated that the greater the distance between the latrine and the water point, the lower the risk of contamination. He also stated that, if the time taken for a pathogen to be transferred to the water point is long, the pathogen would have died off and that the water would no longer be a threat to public health.

The high prevalence of antibiotic resistant bacteria in Nigeria and other developing countries has been associated with several factors including indiscriminate use due to unregulated access of non-professionals to different classes of antimicrobial over-the-counter (Okeke *et al.,* 1999). Tetracycline which has the highest resistance in this study is one of the most commonly available for use as growth promoter and routine chemoprophylaxis for livestock in Nigeria. They are

readily available in different dosage forms and in combination with other antibiotics and vitamins (Jonathan and Afe, 2012).

Results from this study indicated that ceftazidime, chloramphenicol, ofloxacin and ciprofloxacin (flouroquinolones) are the drugs of choice in the treatment of *E.coli* O157:H7 since none of the isolates were resistant to them except ciprofloxacin with a reduced sensitivity 71%. This shows the effectiveness of flouroquinolones, and is in agreement with the findings of Scheld, (2003), suggesting the use of this class of antibiotics. Resistance of *E.coli* O157:H7 isolates to augmentin, tetracycline and cotrimoxazole is similar to a result obtained by Moses and Mopelola (2010) in Ogun State, Nigeria. Resistance to such antibacterials may be due to their wide application in dairy operations.

In this study, *E.coli* O157:H7 isolates were resistant to gentamicin and tetracycline which was contrary to the findings of Daood(2007) in Isfahan, Iran as he recorded susceptibility of the isolates to the antibacterials. Daini and Adesemowo, (2008) also found the resistance of *E. coli* from Nigeria in 54% and 88% against gentamicin and tetracycline respectively. This may be due to the fact that in Nigeria, gentamicin and tetracycline were considered as first line (conventional) drugs used in the treatment of infections caused by *E.coli* O157:H7.

Chloramphenicol has 100% antibacterial activity against *E. coli* O157:H7. This is at variance with the results of Jonathan and Afe (2012) who reported 88.2% resistance to the antibacterial. This may be due to the fact that the antibacterial is not abused and its use has been reduced or nearly stopped and for this reason the drug may have regained its effectiveness (Olatoye, 2010).

Resistance to cefuroxime and gentamicin was 86% each in this study, which was higher than the 65.7% and 11.4% to the antibacterials respectively as obtained by Olatoye (2010) in Ibadan,

Nigeria. This increase in the resistance may be due to the excessive use of the drugs in food animals.

High rate of resistance to sulphamethoxazole-trimethoprim (85%) is in agreement with the previous work by Shroeder *et al.* (2001), who reported that among 189 *E.coli* O157:H7 isolates recovered from various sources between 1985 and 2000, 19 (10%) were resistant to this antibacterial. This is also in line with the work of Reuben and Owuna (2013). They isolated *E.coli* O157:H7 from fermented cow milk (Nono) samples in Nasarawa, Nigeria, among the isolates recovered (84.2%) were resistant to sulphamethazole-trimethoprim. This may be due to the fact that the antimicrobial is commonly used to treat respiratory infections, diarrhoea, mastitis, and other infections in beef and dairy cattle.

Farina *et.,al* (1996) and Kim *et.,al* (2003) concluded that resistance of *E.coli* O157:H7 isolates to tetracycline and sulphamethaxole-trimethoprim are on the increase.

However, ciprofloxacin resistance rate was low as observed in this study, as earlier reported in Turkey by Goncuoglo *et al., (*2010).

For *Salmonella* isolates, all the serotypes were susceptible to fluoroquinolones except *S.* Typhi which shows reduced susceptibility of 66.7% to ciprofloxacin. The high susceptibility of the serovars to fluoroquinolones and cefoxitin as recorded in this study may be due to the high cost of the antibiotics and they are injectables (Akinyemi *et al.*,2007). Therefore, fluoroquinonolones are not indiscriminately abused. The result is similar to a report by Campos *et al.*(1990), Sonstein and Burnham(1993) and Abdullahi *et al.*(2013)

Likewise, susceptibility to third generation cephalosporins as found in this research work is in agreement with the findings of Farhan *et al*.(2012) in Karachi, Pakistan.

Result of this study is at variance with the data obtained from Pakanbaru in Sumatra Island, 2009-2010 in the susceptibility patterns of *S*. Typhi. Anggraini (2010) reported that *S*. Typhi isolates were all susceptible to tetracycline, cefotaxime, ceftazidime, and ciprofloxacin. This marked difference may be due to conditions such as geographical location of the two distinct study areas, disease control programs, local conditions that may include personal hygiene, availability of potable drinking water, food handling and sanitation.

The result of this study was also contrary to reports by Campos *et al*. (1990), Sonstein and Burnham (1993) who reported high susceptibility of *Salmonella* isolates to cefotaxime and ceftazidime. This trend is of particular concern because the extended spectrum cephalosporins are the antibiotics of choice for children (Weill *et al.*, 2004).

Resistance to ceftazidime, cefotaxime, tetracycline, and to a laser extent ciprofloxacin was observed in this study. The resistance could be as a result of the inclusion of preventive doses of antimicrobial agents in poultry feed as growth promoters and treatment which is often associated with the development of resistance in enteric bacterial flora of poultry. These resistant bacteria contribute to the reservoir of resistant bacteria found in the human intestinal tract including resistant *Salmonella* (Dupont and Steele, 1987) The use of antimicrobials for growth-promotion, prophylaxis and treatment of animal's food increases the prevalence of resistance in human pathogens, particularly non typhoidal salmonellosis usually subside without antibiotics treatment especially in immune competent patients. For this reason, the implicated organisms (non-typhoidal *Salmonellae*) are not usually exposed to these antibiotics as compared to *S*. Typhi.

There was an observed reduced susceptibility of *S*.Typhi to fluoroquinolones (ciprofloxacin) as compared to Asia, United Kingdom and some other European countries where resistance to fluoroquinolones have been recorded most likely due to approval of these drugs for use in animals

(WHO, 1998). There was also a report of reduced susceptibility of *Salmonella* spp. to fluoroquinolones in Lagos by Akinyemi *et al*. (2007).

In this study, it was observed that 100%(7) *E. coli* O157:H7 showed multiple antibiotic resistance, 28.6%(2 isolates) were resistant to 5 different antimicrobials, 42.9%(3 isolates) were resistant to 6 antimicrobials, 14.0%(1 isolate) was resistant to 7 antimicrobials and 1 was found be resistant 8 different antimicrobials. The multiple antibiotic resistance index (MARI) was calculated and the isolates all showed MARI of $\geq$ 0.41. While the (MARI) calculated for *Salmonella* serovars was$\geq$0.38. An observation of MARI $\geq$0.38 and 0.41 for *E.coli* O157:H7 and *Salmonella* respectively depicts the source as high risk source, and that, the antibiotics are in constant abuse, also, the act is bringing about high selective pressure (Suresh *et al*., 2000).

# CHAPTER SIX

## 6.0 CONCLUSION AND RECOMMENDATIONS

### 6.1 Conclusion

Groundwater, particularly private and public hand-dug wells supply drinking water for more than half of the Nigerian population. High coliform and aerobic plate counts 20.7 and 3.47±0.02 cfu/ml respectively obtained in this study indicated that, the population of people obtaining drinking water from these wells are at risk of water borne diseases.

The high prevalence of these enteric bacteria in the water could be due to poor sanitary habits of members of the community, the habit of rearing cattle (principal reservoir of *E.coli* O157:H7) and other ruminants within the compounds and also citing of wells close to pit laterines.

Result for the physicochemical analyses of the well water samples showed that, the temperature (18-22$^0$C) and pH (6.9-7.3) of the water samples were within the WHO range, however, high turbidity was also observed in some samples.

*E.coli* O157:H7 and *Salmonella* species (*S.*Typhi, *S.*Typhimurium and *S.*Paratyphi A) were isolated from the well water sampled. It was generally observed that wells cited close to pit laterines were the wells positive for the presence of these pathogenic bacteria. Significant statistical association was also found between the distance of wells to pit laterines and occurrence of these bacteria. The strength of the association (Odds ratio) was further determined and that of *E.coli* O157:H7 and *S.*Typhi were the strongest.

Antibiotics are used extensively to prevent or treat microbial infections in human and veterinary medicine. Results of the present study showed a multidrug resistance of these pathogenic bacteria against the tested antimicrobials. High prevalence of resistance to most of the antibiotics used was

observed, tetracycline and augmentin 100% each, 86% resistance to cefuroxime, gentamicin and sulphametazole-trimethoprim while cefixime had 72% resistance in *E.coli* O157:H7, while ceftazidime, chloramphenicol, ciprofloxacin and ofloxacin proved to be more effective against this pathogenic bacteria. On the other hand, all the *Salmonella* isolates were 100% resistant to ceftazidime, cefotazime and tetracycline and in addition *S.*Typhi isolates showed 33.3% resistant to ciprofloxacin, all the isolates were susceptible to cefoxitin, ofloxacin and chloramphenicol. *S.*ParatyphiA and *S.*Typhimurium were also sensitive to ciprofloxacin.

Multiple antibiotic resistance indices (MARI) was calculated, *E.coli* O157:H7 and *Salmonella* isolates showed MARI of $\geq$ 0.41 and $\geq$0.38 respectively. An observation of MARI $\geq$ 0.41 and $\geq$0.38 depicts the source as high risk source and that, the antibiotics are in constant abuse in the study area.

The development of resistance to antimicrobials occurs through stable genetic change heritable from generation to generation through specific mechanisms including mutation, transduction, transformation, and or conjugation.The shedding of the resistant bacteria into the environment by cattle and other ruminants may lead to a wide spread dissemination of antibiotic resistant genes to the resident bacteria in the environment.

The present results is a warning signal to all stakeholders in community health in the study location to direct action at protecting drinking water from faecal contamination and limiting persistence of antimicrobials in groundwater. The data also suggest that selection pressure imposed by the use of these antibiotics whether therapeutically in human and veterinary medicine or prophylactically in the animal production, is a key driving force in the selection of antimicrobial resistance in organisms.

### 6.2 Recommendations

(i) Prompt well water quality assessment should be undertaken by Katsina State rural water and sanitation agency (KT-RUWASSA)

(ii) The public health workers should ensure that the distance of pit latrine to shallow wells in the study areas meet the recommended distance of 30 m by WHO(2007).

(iii) Katsina State Government should ensure adequate and efficient public water supply through the provision of pipe borne water.

(iv) Public enlightenment campaign on the effect of contaminated water and the danger of citing shallow wells close to pit latrines should be embarked upon by the government and the media (print and electronic), schools, religious bodies e.t.c

(v) A more mechanized method of construction is necessary for a cleaner and tidy well as found for boreholes.

(vi) There is need for Judiciary bodies to legislate and enforce laws to limit the prescription and dispensing of antibiotics and other drugs to only qualified professionals.

(vii) Provision of protective covers to the wells can also minimize the possibility of contamination especially during rainfall, and storm events.

(viii) There is the need to develop cheap and effective small-scale water disinfection methods for making well water safe for drinking, thus the appropriateness of water storage, solar water disinfection, boiling and chlorination as points of use (small-scale) methods for making well water safe for drinking.

(ix) Improved sanitary conditions should be encouraged to avoid introduction of contaminants into the wells.

(x) There is need for the government to enforce the existing laws that prohibit smuggling of all kinds of animal products such as meat, poultry and their products so as to overcome the problems of multi-drug resistant organisms.

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## APPENDIX 1

**Table 2.1: Summary of Largest *E. coli* O157:H7 Outbreak Cases in Some Developed Countries.**

| Year | Place | Number of People Sick | Source |
|---|---|---|---|
| 1989 | Montana, US | 243 | Undercooked Ground Beef |
| 1996 | Sakai, Japan | 5,727 | White radish prout |
| 1996 | Scotland, UK | 496 | Undercooked Ground |
| 2000 | Walkerton, Canada | Over 2000 | Contaminated Drinking Water |

| | | | |
|---|---|---|---|
| 2002 | Pennsylvania, US | 51 | Infected Dairy |

Table 2.2: Summary of *E. coli* O157:H7 Outbreak Cases in African Continent Since 1990

| **Year** | **Place** | **Number of People Sick** | **Source** |
|---|---|---|---|
| 1990 | South Africa | 1 | Unknown |
| 1992 | Swaziland | 40,192 | Beef and Water |
| 1992 | Kenya | 1 | Unknown |
| 1997 | South Africa | 151 | Water |
| 1998 | Cameroon | 298 | Water |

| | | | |
|---|---|---|---|
| 2003 | DRC | 463 | Water |
| 2003 | CAR | 463 | Zebu meat |
| 2003 | Nigeria | 187 | Water |

## APPENDIX 2

**Table 4.5a: Colonial characteristics, Gram reaction and Biochemical Identification of *E.coli* O157:H7**

| Sample location | Appearance on EMB Agar | Gram Reaction | Cell Morphology | Indole | Methyl-Red | Voges Proskauer | Citrate utilization | Oxidase | Urease | Motility | L.D.C. | CHO Fermentation | | | | Identification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | Lactose | Mannitol | Sucrose | Glucose | |
| **Mn79** | **GMSC** | **Gram –** | **Short rods** | + | + | - | - | - | - | + | + | + | + | + | + | ***E. coli*** |
| **Mn84** | **GMSC** | **Gram –** | **Short rods** | + | + | - | - | - | - | + | + | + | + | - | + | ***E. coli*** |
| **Dm3** | **GMSC** | **Gram –** | **Short rods** | + | + | - | - | - | - | + | + | + | + | + | + | ***E. coli*** |
| **Dm21** | **GMSC** | **Gram –** | **Short rods** | + | + | - | - | - | - | + | + | + | + | + | + | ***E. coli*** |
| **Dm38** | **GMSC** | **Gram –** | **Short rods** | + | + | - | - | - | - | + | + | + | + | + | + | ***E. coli*** |
| **Dm47** | **GMSC** | **Gram –** | **Short rods** | + | + | - | - | - | - | + | + | + | + | + | + | ***E. coli*** |
| **Dj9** | **GMSC** | **Gram –** | **Short rods** | + | + | - | - | - | - | + | + | + | + | + | + | ***E. coli*** |

**Keywords: += Positive,- = Nagetive, ,Mn= Mani, Dm= Dan-musa ,Dj= Danja, GMSC= Green Metallic Sheen Colonies. L.D.C=LysineDecarboxylase.**

**Table 4.5b Colonial characteristics, Gram reaction and Biochemical Identification for *Salmonella* Isolates**

| Sample location | Appearance on SSA | Gram Reaction | Cell Morphology | CHO Fermentation | | | | Urease | Oxidase | Motility | L.D.C | Citrate utilization | Indole | Slope | Butt | $H_2S$ | Gas | Identification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Glucose | Sucrose | Mannitol | Lactose | | | | | | | | | | | |
| **Mn6** | **CCCBC** | **Gram -** | **Rods** | + | - | + | - | - | - | + | + | - | - | **R** | **Y** | + | + | ***Salmonella*spp.** |
| **Mn22** | **CCCBC** | **Gram –** | **Rods** | + | - | + | - | - | - | + | + | - | - | **R** | **Y** | + | + | ***Salmonella*spp.** |
| **Mn67** | **CCCBC** | **Gram –** | **Rods** | + | - | + | - | - | - | + | + | - | - | **R** | **Y** | + | + | ***Salmonella*spp.** |
| **Mn69** | **CCC** | **Gram –** | **Rods** | + | - | + | - | - | - | + | + | - | - | **R** | **Y** | - | + | ***Salmonella*spp.** |
| **Dm5** | **CCCBC** | **Gram –** | **Rods** | + | - | + | - | - | - | + | + | - | - | **R** | **Y** | + | + | ***Salmonella*spp.** |
| **Dm53** | **CCC** | **Gram –** | **Rods** | + | - | + | - | - | - | + | + | - | - | **R** | **Y** | - | + | ***Salmonella*spp.** |
| **Dm75** | **CCCBC** | **Gram –** | **Rods** | + | - | + | - | - | - | + | + | - | - | **R** | **Y** | + | + | ***Salmonella*spp.** |
| **Dm82** | **CCCBC** | **Gram –** | **Rods** | + | - | + | - | - | - | + | + | - | - | **R** | **Y** | + | + | ***Salmonella*spp.** |
| **Dj11** | **CCCBC** | **Gram –** | **Rods** | + | - | + | - | - | - | + | + | - | - | **R** | **Y** | + | + | ***Salmonella*spp.** |
| **Dj66** | **CCCBC** | **Gram –** | **Rods** | + | - | + | - | - | - | + | + | - | - | **R** | **Y** | + | + | ***Salmonella*spp.** |
| **Dj69** | **CCC** | **Gram –** | **Rods** | + | - | + | - | - | - | + | + | - | - | **R** | **Y** | - | + | ***Salmonella*spp.** |

**Key: + Positive, - Negative, R-Red-pink(Alkaline reaction), Y-Yellow(Acid reation), Mn- Mani, Dm- Dan-musa, Dj- Danja, CCC- Cream Coloured Colonies, CCCBC- Cream Coloured Colonies with Black centres,**
L.D.C=LysineDecarboxylase

## APPENDIX 3

### Control Strategies to Prevent *Salmonella* and *E.coli* O157:H7 in Drinking Water

Different measures can be adopted to hinder the spread of *Salmonella* and *E.coli* O157:H7 in water environment, and consequently reduce human risk of infection. Regarding the measures to reduce the occurrence of these enterobateriaceae in drinking water, the control strategies have to be elaborated and adopted on the basis of the water resource availability and the economic and developmental level of the country. For this purpose, the World Health Organization has developed Guidelines for Drinking Water Quality, which provides an internationally harmonized support to help countries develop rules and standards that are suitable to national and local circumstances (WHO, 2008).

In developing countries, commonly lacking piped water supply, self-sustaining decentralized approaches including point of use chemical and solar disinfection, safe water storage and behavioral changes are indicated as reliable options to directly target the most affected population and reduce water-borne disease burden through improved drinking water quality (Mintz *et al.*,2001). In this respect, three recent reviews that used meta-analysis to evaluate the effect of water treatment at point of use on water quality and diarrhea reduction showed that point of use interventions more efficiently reduced diarrhea incidence, with respect to water source interventions (Arnold & Colford, 2007).

Studies of the risk factors for diarrhea, typhoid and paratyphoid endemics and epidemics infections confirm that simple decentralized control strategies might be effective in the control of these diseases (Sharma *et al.,* 2009; Srikantiah *et al.,* 2007). According to the findings of Sharma *et al*. (2009) home chlorination of drinking water, the use of narrow-mounted containers for safe water storage, and drawing out water by tilting the container or using taps to avoid contamination

may be effective practices to avoid spread of diarrhea and typhoid in west Bengal India. Farooqui *et al*. (2009).

Reported measures recently introduced by WHO that include solar disinfection, bleach addition, boiling, and use of low cost ceramic filters, which are well suited to prevent diarrhea and typhoid fever outbreaks due to the consumption of contaminated surface drinking water.

Education regarding the risk of drinking untreated surface water as well as promoting the use of boiled drinking water carried from home was indicated by Srikantiah *et al*. (2007) as practical intervention measures to reduce water related transmission of typhoid fever in Samarkand (Uzbekistan). Srikantiah *et al.* (2007) also suggested specific intervention strategies to reduce the increased risk linked to MDR typhoid strains. According to their results the use of antimicrobials two weeks before onset of typhoid fever was independently associated to this disease; hence the development of primary care clinical treatment guidelines to reduce unnecessary antimicrobial exposure may efficiently decrease the risk of diarrhea and typhoid fever In many industrialized countries, the success of applied control strategies is confirmed by the small number of water-borne outbreaks caused by *salmonellae*. Nevertheless, outbreaks caused by microbial contamination of drinking water still result in substantial human and economic costs in these countries (Berg, 2008; Risebro *et al*., 2007).

The identification of the causes of outbreaks and the potential threats to water quality is necessary to formulate effective prevention strategies that will minimize the further onset of outbreaks and related costs.

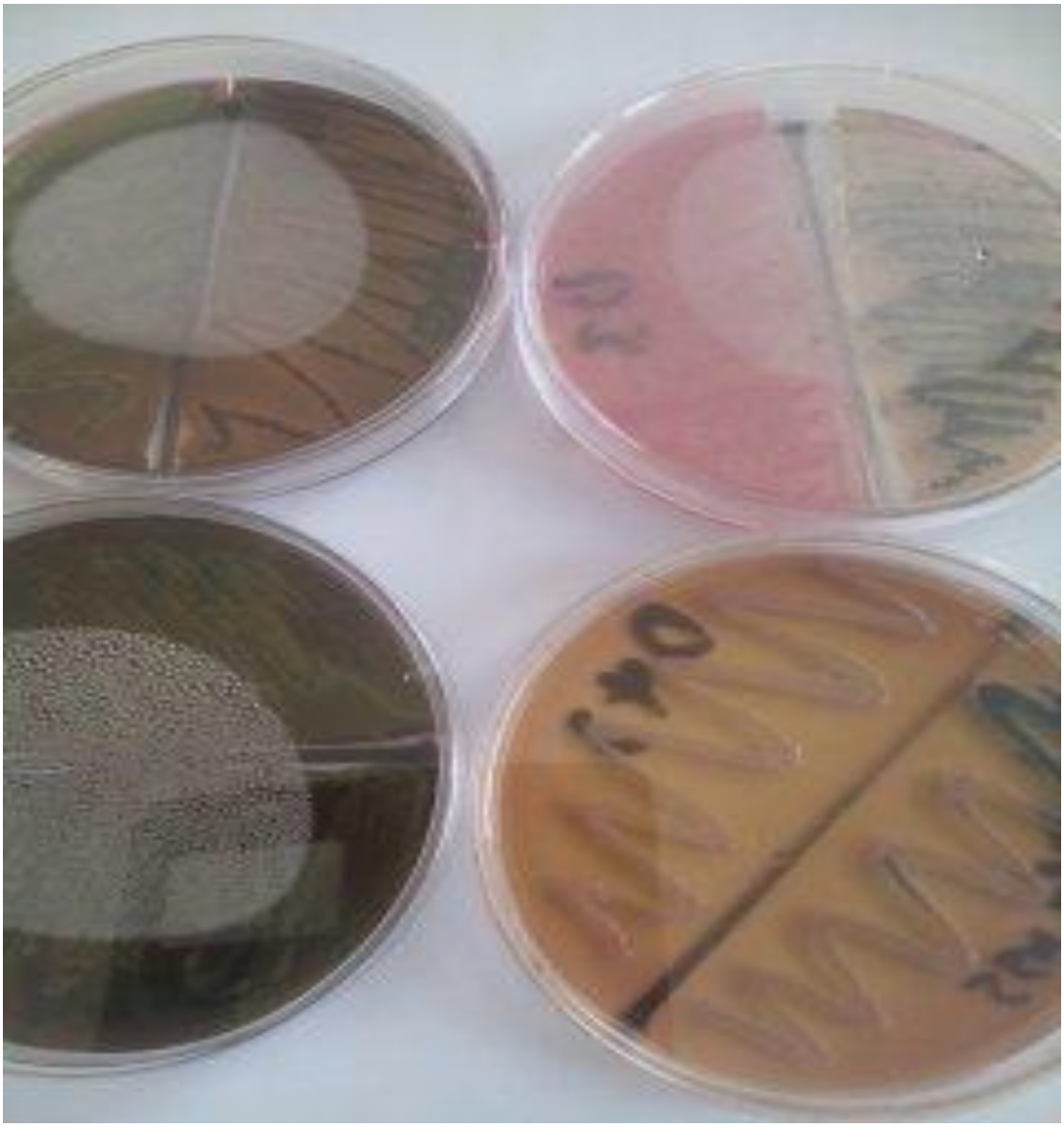
## APPENDIX 4


DISTANCE 3 METERS


Dirty kitchen wares
around the well


Goat being reared 3m
away from the well


Dirts and faecal materials close to the well




Distance 3 meters